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Full Name of Author — Nom complet de l'auteur

Beverley Ann Ross

Date of Birth — Date de naissance

Aug. 21, 1945

Country of Birth — Lieu de naissance

Canada

Permanent Address — Résidence fixe

#306-8510-111St
Edmonton, Alberta
T6G-1H7

Title of Thesis — Titre de la thèse

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Name of Supervisor — Nom du directeur de thèse

Dr. G.R. Webster

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TOLERANCE OF *Hylocomium splendens*, *Ptilium crista-castrensis* and *Pleurozium schreberi* TO SALT SHOCK CAUSED BY BRINE SPILLS

by

(C)

BEVERLEY ANN ROSS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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(SIGNED) Beverley Ross

PERMANENT ADDRESS:

#306-8510-111 Street

Edmonton, Alberta

T6G-1H7

DATED April 24 1984

THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **TOLERANCE OF** *Hylocomium splendens, Ptilium crista-castrensis and Pleurozium schreberi*

TO SALT SHOCK CAUSED BY BRINE SPILLS submitted by BEVERLEY ANN ROSS in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

G.R. Wilson
Supervisor

D.H. Vitt

J.A. Robertson

Date April 16, 1984

Köszönöm szépen

Gracias

谢谢你

Merci

Dank

Asante

شكرا

有美匯

Dedicated to
International Students

Thank you

Danke

Ndikomo

Me da wo ase

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Dalu nu

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Dziękuje

Med hjertelig takk
til min familie.

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ABSTRACT

The inception of this study was a project on reclamation of brine spills in upland boreal forest areas. During a brine spill flora are subjected to a salt shock because of the sudden increase in salt concentration. Six greenhouse experiments investigated the effects of brine and post flush treatments on three feather mosses. Namely *Hylocomium splendens*, *Ptilium crista-castrensis*, and *Pleurozium schreberi* which are common to coniferous forests in Alberta.

Study objectives were:

1. To develop laboratory methods to measure moss response to brine and reclamation treatments;
2. To determine if the osmotic plus specific ion effects of brine were more harmful than the osmotic effect alone;
3. To compare the effects of brine exposure time, versus salt concentration on moss regrowth;
4. To ascertain if calcium and/or potassium would aid moss recovery;
5. To gain insight into some physiological aspects of the three feather mosses.

The most satisfactory procedure is described below. Green moss segments were wrapped with nylon net to form a bundle. Simulated spills of distilled water, brine, or sorbitol were then applied. Bundles were drained, flushed with distilled water, and post flush treatments given where applicable. Mosses were then placed in a horizontal

position, in a plastic propagation tray, for recovery in the greenhouse. Throughout the recovery period, the mosses were misted with a 1/20 strength nutrient solution. Response was measured by clipping and weighing moss regrowth.

Osmotic plus specific ion effects of brine were more harmful to moss regrowth than the osmotic effect of sorbitol. Mosses were more tolerant to drought than salt. *Ptilium* was the least drought tolerant and *Hylocomium* was the least salt tolerant species. *Pleurozium* appeared to be the most salt tolerant moss in the first set of experiments. However response of *Pleurozium*, compared to the other two species, changed in the next two experiments.

In terms of damage, salt concentration of brine was more important than exposure time. The 'Species X Concentration' interaction was highly significant ($P \leq 0.01$), but 'Species X Exposure Time' was not significant. If these findings hold true under field conditions, it would be advantageous to flush a spill site as quickly as possible to lower the brine concentration. In treatments with maximum brine exposures and concentrations, *Ptilium* gave the highest amount of regrowth of the three species.

Under the given conditions, calcium or potassium did not aid moss recovery. There were no significant differences in any calcium/potassium post flush treatments. Differences among moss species were highly significant. *Ptilium* had the greatest amount of regrowth and *Pleurozium* the least. The response of *Ptilium* and *Pleurozium* was reversed to that of

earlier experiments. Whether relative humidity or time of year influenced moss response, remains for future investigation.

Response of *Pleurozium* varied the most in the six experiments. When brine and sorbitol were absent, *Hylocomium* produced the most and *Ptilium* the least biomass. *Hylocomium* turned brown quickly under humid storage conditions while *Ptilium*, the least drought tolerant species, was the last species to discolor. Therefore *Hylocomium* may be the most drought tolerant species tested. Invader moss species favored *Ptilium* for colonization. The regeneration capabilities of mosses should be investigated further as moss spores or fragments could be used to 'seed' spill sites and thus reduce soil erosion.

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Me da wo ase.

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1. INTRODUCTION

In the petroleum industry one usually associates the word 'spill' with 'oil spill', however there is another spill that is becoming more prevalent - the 'brine or salt water spill'. As oilfields age the ratio of salt water to oil, brought to the surface, increases and since brine is the most corrosive fluid in the petroleum recovery system, the potential for brine spills to occur also increases with time.

Salt water spills in forested areas have increased in the past few years with increased petroleum activity in these areas (Webster *et al*. 1983). High rainfall, hilly terrain, and physical properties of forest soils are some factors that lead to severe erosion hazards when plant cover is removed as a result of a brine spill. Appropriate reclamation of affected sites can reduce or prevent erosion of the land. In the past, reclamation techniques designed for agronomic areas were applied to forested areas but there is evidence that different reclamation procedures are needed for forested land. For example, seeding a grass cover on a large disturbed area made it impossible to establish trees because the grass cover favored a greatly increased population of gnawing rodents (Sherstabetoff *et al*. 1978). Most agronomic systems are based on a monoculture of a particular species or more specifically a particular cultivar, except in a hay or pasture regime where there is a mixture of legumes and grasses. However in the forest system

one deals with a multi-layered plant system of wild species, from trees to mosses, that represent a wide range in plant morphology and physiology, plus animals of the forest ecosystem.

To formulate procedures to reclaim brine spills in upland forested areas, experiments were conducted at two sites in the Swan Hills from 1977-1981 (Innes and Webster 1980a). One of the greenhouse studies, that complemented the Swan Hills field investigation, was a preliminary project on moss/brine relationships (Ross Daley 1980). This work was an introduction to the area of moss/salt interactions. Exposure to brine for short periods of time e.g. three days, tests the *Salt Shock Tolerance* of plants which is different from Salt Tolerance (where a plant grows at a relatively constant level of salt concentration throughout its life (Ross and Webster 1983).

The present study of feather moss/salt relationships involved samples of the three prevalent moss species (Hettinger 1980) from the two Swan Hills sites: *Hylocomium splendens*(Hedw.)B.S.G., *Ptilium crista-castrensis*(Hedw.)De Not. and *Pleurozium schreberi*(Brid.)Mitt. The study had the following objectives:

1) To develop laboratory methods that would effectively test and measure the response of three feather mosses to the various brine and reclamation treatments.

2) To determine whether the osmotic plus specific ion effects of brine were more harmful to feather mosses than the osmotic effect alone.

3) To ascertain degree of damage to the mosses by an increase in brine contact time compared to an increase in the electrical conductivity of the brine, in a surface response factorial.

4) To determine if moss recovery can be aided by post flush treatments of calcium, potassium or a combination of the two cations.

5) To learn more about the basic physiology of the three feather mosses by comparison of their response patterns to various brine and reclamation treatments.

2. LITERATURE REVIEW

2.1 BRINE

Wells for production of salt water were in operation in the U.S.A. as early as 1800, fifty nine years before Colonel Drake brought in the first oil well in Pennsylvania (Tiratsoo 1976). Salt water operators were not happy with the discovery of oil in some of their operations and not knowing what to do with the oil, they often moved to new locations to avoid the 'messy nuisance' which spoiled their operation. Today salt water is the messy nuisance that oil well operators must handle (Collins 1975). If a spill occurs the salt water becomes the concern of the reclamationist.

Haynes *et al.* (1978) expressed concern about environmental impacts of enhanced oil recovery. Four of the points stated apply to petroleum operations in general and concern brine, vegetation or land:

1. "pollution of land and surface waters by spills or leaks of oil, brine or other chemicals
2. contamination of ground water aquifers by injected chemicals, oil or brine through communications between active wells, abandoned wells and inadequately plugged wells
3. excessive erosion and sedimentation (mostly in hilly terrain) and subsequent deterioration of surface water quality
4. loss of vegetation (land disturbed is roughly a third of

the total area used in the water flooding pattern of enhanced recovery)."

Acceptable monitoring, preventive and reclamation procedures are available for most environmental problems related to enhanced oil recovery, but the best available techniques may not be known by operators nor required by law (Haynes *et al.* 1978). Petroleum operators should know something about the environmental impact of their operations, likewise salt spill reclamationists should have some basic knowledge of petroleum operations in order to research, solve or reduce the environmental problems of brine spills. A few basic facts about the system would also enable reclamation personnel to reduce the number of treatment possibilities. They could make some general plans rather quickly, especially if laboratory facilities are not available and time is of the essence. Webster (1975) stated "the best practice is to prevent spills."

When planning my experiment I was unaware of the various chemicals added to the formation water during the petroleum recovery process therefore it was assumed sodium chloride was the dominant toxic compound in the brine. Only the main inorganic salts of one brine sample were considered in the study. It remains to be determined what deleterious effects the organic components, hydrogen sulfide or various additives (corrosion or scale inhibitors, gas scavengers, bacteriocides, enhanced recovery chemicals or cleaning agents) may have on forest vegetation. Background

information is presented to answer some of the other questions raised.

1) Where do brines originate and are all brines the same?

2) Why do spills occur and what is spilt?

3) Can spills be prevented?

4) What is the potential for future brine spills?

2.1.1 ORIGIN AND COMPOSITION OF BRINES

Most deposits of petroleum and natural gas are associated with sedimentary rocks of marine origin (Leet *et al.* 1978). Organic matter from land and marine plants and animals is deposited with inorganic sediments to form a organic material from which petroleum and natural gas originate. Water influences not only the hydrocarbon precursors but the generation, migration, accumulation, alternation and production of petroleum. Accumulation and production depend upon hydraulic flow in response to geostatic and hydrostatic pressure (Collins 1975).

When water evaporates, under restricted environmental conditions, deposition of salts occurs. Collins (1975) stated the common order of salt deposition in a basin cut off from the open sea is:

"Calcite-$CaCO_3$>dolomite-$CaMg(CO_3)_2$> gypsum-$CaSO_4.2H_2O$> halite-NaCl>sylvite-KCl> carnallite-$MgCl_2.KCl.6H_2O$> bischoffite-$MgCl_2.6H_2O$"

Oilfield brines however, are not formed by simple evaporation or dilution of sea water alone (Kramer 1969; Collins 1975). Modifications may take place by dilution from ground water, ion exchange, precipitation of minerals, solution of surrounding rock and selective membrane filtration (Rittenhouse 1967; Kramer 1969; Billings *et al.* 1969). Collins (1975) stated that the composition of *seawater* may vary somewhat, but in general will have a composition relative to the following:

TABLE 1: Average composition of seawater

	mg/L(ppm)
Chloride	18,980
Sodium	10,560
Sulfate	2,710
Magnesium	1,270
Calcium	400
Potassium	380
Bromide	65

Oilfield brines may contain much higher concentrations of dissolved salts than that of seawater; concentrations of 220,000 mg/L(ppm) dissolved solids are common (Ostroff

1965). Brines are basically sodium, calcium, and magnesium chlorides. They are deficient in magnesium and sulfate but have excess calcium and carbonates relative to seawater (Kramer 1969).

Rittenhouse *et al.* (1969), examined the minor elements in 823 oilfield brines from the U.S.A. and Canada, and their findings are as follows:

"Potassium and strontium 100+ mg/L(ppm);
Aluminum, boron, barium, iron, lithium and silicon 1-100 mg/L(ppm);
Chromium, copper, manganese, nickel, tin, and zirconium in parts per billion(ppb) in most brines;
Beryllium, cobalt, lead, vanadium, tungsten and zinc in parts per billion(ppb) in some brines."

To compare brines with seawater, differences in total dissolved solid contents are eliminated by calculating concentrations on the basis of 35,000 mg/L(ppm). "Chromium, lithium, manganese, silicon and strontium are commonly more than twice as abundant as in seawater. Copper, potassium, nickel, and tin are commonly less than half as abundant as seawater" (Rittenhouse *et al.* 1969). Potassium tends to be adsorbed from the brine and fixed by clay minerals, mica and potassium feldspars; boron is released by the minerals into the brine (Grim 1968).

Oilfield waters are referred to by various names such as brines, salt waters, waters, or formation waters. *Formation water* is defined by Case (1955) as water that

naturally occurs in the rock and is present immediately before drilling. Formation waters or brines are often grouped together on the basis of their inorganic ions, the geological age of the formation, or by the specific geological zone. Bentor (1969), classified subsurface brines in Israel into four different groups which demonstrate some of the main variations in brines.

Group 1 Composition and salinity identical with present day seawater, except for higher calcium and lower magnesium levels, a change attributed to dolomitization.

Group 2 Early Cretaceous water is two or three times more saline than seawater. Most of the sulfates were lost through reduction to hydrogen sulfide by sulfate reducing bacteria (Ostroff 1965). Brines are enriched in bromide and iodide from bioconcentrators such as seaweed and coral (Collins 1975).

Group 3 Brines from Jurassic to Early Cambrian sources progressively increased salinities and the Ca/Na and Br/I ratios with depth whereas Na/K, HCO_3/Cl and Cl/Br ratios decreased. Brines changed from NaCl to $CaCl_2$ types by surface evaporation and later modified in the subsurface by differential ultrafiltration.

Group 4 A highly saline brine at the depth in the Rift Valley was a $CaCl_2$ type with Ca/Na ratios greater than one. It was divided into two subgroups, the first a highly differentiated Early Paleozoic brine. The second was an old Paleozoic brine with salinities around 300,000 mg/L(ppm), a Na/K ratio of one to two and extreme $CaCl_2$ character. It was formed by an additional cycle of surface concentration through evaporation.

Rittenhouse (1967) classified brines into five groups on the basis of their bromide content and all five groups of formation waters are present in the Western Canada sedimentary basin (Billings *et al*. 1969). Gilbart (1952) studied the analyses of Alberta brines and presented the results on the basis of geological formations. He classified the waters of the Southern Plains into six broad groups called water horizons.

Samples used in the present experiment were battery samples from the Beaverhill Lake Formation in the Swan Hills field. There is no gas cap or major oil/water contact in this formation and the average porosity is 7.9%, with a connate water content of 16% (Edie 1961; Cameron 1968) and hydrogen sulfide was absent (Van Everdingen 1968). Analyses of 'well head' samples (opposed to battery samples) from the various fields of the Beaverhill Lake formation and adjacent zones show a range of ion concentrations, pH and electrical conductivity (Table 2).

TABLE 2: Analyses of wellhead brine samples (mg/L(ppm))

FIELD	ZONE	Cl	Na	Ca	Mg	SO_4	HCO_3	pH	E.C.[1]
Swan Hills	Beaverhill Lake	125,400	68,152	11,692	1,118	621	490	6.1	195
Swan Hills	Beaverhill Lake	120,658	61,996	11,913	1,533	426	280	6.5	149
Swan Hills	Beaverhill Lake	111,825	54,395	12,680	2,090	635	454	7.6	233
Swan Hills	Beaverhill Lake	111,718	66,190	4,930	573	639	192	6.5	169
Swan Hills South	Beaverhill Lake	101,600	56,970	7,047	778	463	1,162	6.2	166
Swan Hills	Gilwood	132,000	62,757	16,125	2,338	150	75	6.5	196
Swan Hills	Winterburn	93,000	44,151	9,732	2,983	1,164	280	7.1	123
Swan Hills	Basal Quartz	42,097	25,972	1,300	131	280	685	7.8	80
Swan Hills	Pekisko	31,328	19,443	907	240	489	1,045	7.9	66
Swan Hills	Viking	24,800	14,972	569	302	12	297	7.2	58

[1]E.C.=electrical conductivity in mS/cm

2.1.2 OCCURRENCE OF BRINE SPILLS

Brushett (1975) stated, "that in Alberta, pipelines appeared to be the major source of spills with corrosion being the most frequent cause of line failure." In 1974, there were about four times the number of salt water spills as there were oil spills (Edwards and Blauel 1975). Although the main component of a brine spill is formation water, there can be a number of additional chemicals involved. The phytotoxicity of various organic and inorganic chemicals used in the different stages of production must be considered e.g. acids, corrosion inhibitors, scale prevention agents, cleaning agents, gases and enhanced recovery chemicals. Haynes *et al.* (1978) suggested that research into the environmental impact of enhanced recovery techniques is necessary. Important research needs are:

1. characterization and toxicity studies of chemicals used in injection techniques and process effluents
2. evaluation of ground water monitoring systems
3. reclamation procedures for soils contaminated by oil and brine.

Spills can occur as oil only, brine only or mixed brine and oil, depending on the location of the fault in the petroleum recovery system (Figure 1). Most spills occur at some point in the disposal or injection system (Thimm 1983, personal communication). Reclamation techniques can vary for each of the different types of spill.

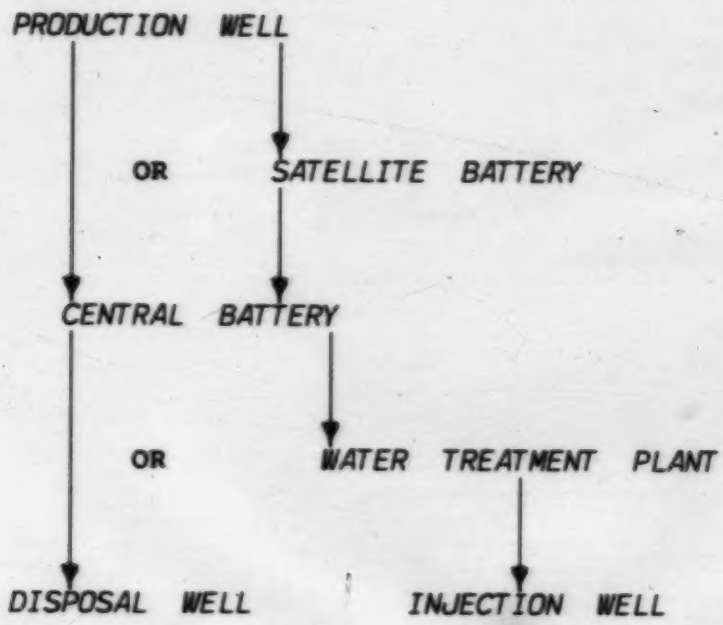

FIGURE 1: Brine in the petroleum recovery system

A mixture of oil, gas and water is forced out of the formation by natural flow, gas lifting, gas repressurization or by pumping methods at the wellhead (DiStasio 1981). Formations are sometimes treated with acids, e.g. hydrochloric, nitric, sulfuric, formic, hydrofloric or acetic, to increase permeability of the reservoir rocks and thus increase the flow input or output (Collins 1975). A gathering system of pipelines or trucks collects production from a number of wells and transports it to a satellite battery for separation of gas from fluids in a two phase separator. If the system does not include satellite batteries, all three components are separated by a three phase separator at the central battery (Skinner 1981). Any spills that take place from the production well to the

central battery are mixed spills of oil and brine (Rowell and Crepin 1977).

Brine is removed from the oil before transport to the refinery by use of such methods as the following: settling tanks, heater treaters, gunbarrel treaters, electrostatic treaters, and chemical separators. A small amount of oil which could cause possible adverse effects to disposal or injection wells, pumps and equipment, may still be left in the water after these treatments. Therefore further treatment may involve mechanical skimmers, aeration skimmers, coalescers, filters or a combination of these methods. If corrosive gases are not a problem and there is no enhanced recovery program, the water is sent to the disposal well. Disposal wells are usually located outside or near the edge of a lease. Storage facilities for the various components are usually located at all phases in the system and trucks or pipelines may be used to transport brine to the disposal sites. For more information consult the American Petroleum Association's 1960 publication.

For many years, the common method of saltwater disposal was to pump the brine into open pits. Evaporation and percolation into the earth's surface removed the water fairly quickly. Open pit disposal has been largely abandoned because of the possibility of danger to human, animal and plant life (Skinner 1981). However restricted use of open pits is still allowed in Alberta. Open pits in Alberta are only allowed under a special Energy Resources Conservation

Board permit and companies are restricted to disposal of one hundred barrels per month (Shaw 1983, personal communication).

Injection wells are used for enhanced recovery of oil by water flooding the formation in a given pattern. In the popular five spot pattern (Figure 2), the production well is in the center of four injection wells (Skinner 1981).

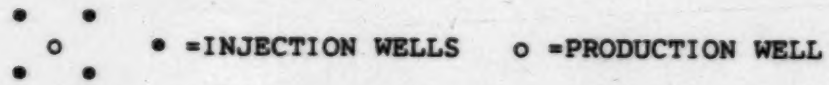

FIGURE 2: Five spot pattern of water flooding for enhanced recovery of oil

Water used for injection wells, usually goes to a water treatment plant as wells can be plugged by entrained solids, oil and bottom settlings, sulfur, bacteria and precipitates of salts. The water treatment plant generally consists of facilities for de-aeration, chemical coagulation, sedimentation, filtration and storage of treated water. Facilities may also include apparatus for the addition of chlorine or some other bactericide. In some systems algae and bacteria may grow in the injection well and plug the formation. If sulfate reducing bacteria are a problem a strong bactericide will probably be necessary to control their growth (Ostroff 1965). Water may be further treated to make it chemically compatible with formation water and also enhanced recovery chemicals added (Skinner 1981). In some cases another source of water, e.g. river or ocean water is

also treated and blended with brine, from the system, before injection (Collins 1975).

2.1.3 PREVENTION OF BRINE SPILLS

Scale formation, corrosion and microorganisms are the main problems in the water handling system (Ostroff 1965; Patton 1977). Ironically gypsum, a 'friend' to salt spill reclamation, is regarded as a 'foe' in the petroleum recovery system. Changes in partial pressures and temperatures, from the formation to the surface, cause precipitation of gypsum and other salts which leads to scale buildup. Scale buildup necessitates removal by physical or chemical means. Some of the chemicals used to inhibit scale formation are EDTA, nitrilotriacetic acid salts, various polymetaphosphates and sodium carboxymethyl cellulose (Ostroff 1965; Collins 1975). Inorganic and organic scale inhibitors, corrosion inhibitors, gas scavengers and bacterial agents are some of the chemical means used to help prevent ruptures.

Prevention of ruptures, begins with the design of the water handling system (American Petroleum Association 1960; Patton 1977). A system needs individual parts that can be isolated without a loss of production, e.g. a number of small plastic or epoxy lined tanks for brine storage, instead of one large one. Distribution systems must be: capable of withstanding the maximum station pressure for extended periods of time, able to transport the fluid with

essentially no pressure loss from friction, and able to withstand the attack of sometimes extremely corrosive fluids for more than twenty years (Skinner 1981).

Metal corrosion by electrochemical and microbiological means is one of the major problems with most water handling systems (Ostroff 1965; Patton 1977; Skinner 1981). Unlike other petroleum production equipment, disposal or injection wells and their associated pipeline, storage and pumping systems seldom contact any liquid other than very corrosive water. This is likely to cause severe corrosion unless care is exercised to prevent it (Skinner 1981). Dissolved salts, pH, temperature, partial pressure, dissolved gases and velocity are some of the factors to consider in corrosion and scale prevention (Ostroff 1965).

De-aeration and degasification of water are used to reduce the presence of oxygen, carbon dioxide, and hydrogen sulfide. These three gases can increase the corrosivity of water. Oxygen is probably the most troublesome corrosion producing substance. In water containing high salt concentrations, corrosion is proportional to the amount of oxygen dissolved in water. The action of oxygen and carbon dioxide together is synergistic. The corrosion rate caused by dissolved hydrogen sulfide is higher in brine than distilled water. Hydrogen sulfide is also highly toxic and certain line cleaning agents tend to generate this gas. Physical or chemical (gas scavengers) means are used to remove these gases (Ostroff 1965; DiStasio 1981).

There are a wide variety of chemical substances used as corrosion inhibitors, both inorganic and organic. Cement, asbestos, plastic and fiberglass are some of the materials used to line distribution lines in the system, especially those from the central battery to disposal or injection wells. Generally organic inhibitors form a film on the metal surface, protecting it from attack. Some inorganic compounds, like chromates, are anodic inhibitors or passivators. Chromates, phosphates, arsenates' and silicates are dissolved in water to inhibit or reduce corrosion by coating the metal surface to protect if from the electrolyte.

Corrosive resistant metal such as copper-nickel or titanium alloys are recommended for pumps and meters in the water handling system. Cathodic protection is applied to protect steel, brass, lead, copper and aluminum against electrochemical corrosion (Ostroff 1965; Skinner 1981). Magnesium is frequently used to provide cathodic protection, but magnesium is also a stimulant to certain classes of bacteria which can create a corrosion problem (Shaw 1984, personal communication).

2.1.4 POTENTIAL FOR FUTURE BRINE SPILLS

With some oil wells there is little or no brine when first put into production, but over time they gradually

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'Shell and some others have voluntarily restricted use of arsenates since 1960 (Shaw 1983, personal communication)

produce more and more brine. As some wells become older, the produced fluids may be more than 95% brine, or for each cubic meter of oil coming to the surface one hundred cubic meters or more of brine is also produced (Collins 1975).

When the ability of conventional methods of primary production to recover oil is exhausted, there still remains a considerable quantity of oil in the formation and in order to recover some of this oil, secondary and tertiary techniques may be used (DiStasio 1981). Over the last ten to fifteen years, research has been conducted to find practical methods of recovering petroleum left in the earth after known recovery methods have been used. Many experts predict that 60% recovery will become commonplace and 90% recovery may be expected in the future (Skinner 1981).

Water disposal will become more critical because the volumes must increase with time. It is normal for water production rates to increase with time in waterflood and enhanced recovery projects (Skinner 1981). The potential for spills to occur will be increased by enhanced recovery techniques such as increased water production, use of high pressure gas injection on equipment intended for low pressure water injection, and use of chemicals and gases which may increase the corrosion rate (Haynes *et al.* 1978; Skinner 1981).

Water injection recovery techniques may vary in detail but broadly most involve water-flooding the formation in order to force the oil towards the production well or wells.

Polymers (e.g. polysaccharides, polyacrylamides and hydroxyethyl cellulose) (Unsal *et al.* 1979) are added to the injected water to increase the 'sweeping' efficiency and broaden the range of water injection (Burger 1979).

In recent years, improved methods of water injection have been developed. Injection of micro-emulsions and surfactants have increased the efficiency of displacement, together with injection of alkaline solutions which reduce surface tension at the oil/water interface or increase the wettability at the rock/water interface (Burger 1979). Surfactant or micellar flooding employs an aqueous surfactant slug followed by a mobility buffer (usually polymer thickened water) to displace oil locked in small pores (Burger 1979).

Carbon dioxide is the main gas used for injection and since it forms a mild acid when mixed with water, it may cause corrosion problems not normally encountered. A method which uses a gas mixture with nitrogen is also being successfully tested. Both hydrogen sulfide and carbon monoxide are used in an in situ combustion technique. Steam injection techniques are used in large-scale production projects to recover oil that is slightly less viscous than hot asphalt (Skinner 1981; DiStasio 1981). The Esso pilot plant at Cold Lake uses the steam injection method.

Regular inspection, maintenance and repair of the recovery system is necessary to reduce corrosion and spills to a minimum. However,

"EVERY PIECE OF EQUIPMENT, NO MATTER HOW WELL CONSTRUCTED, WILL EVENTUALLY FAIL IN SOME WAY."

(Skinner 1981)

Therefore materials should be on hand to deal with a spill effectively and as quickly as possible. Consult the field manual by Innes and Webster (1980b) for further instructions on reclamation of salt spills in upland forested areas.

From 1977 to 1981 a research project to study reclamation of brine spills in forested areas was undertaken at two sites in the Swan Hills, one site was Amoco Oil(LSD 12-36-64-11 W5M) and the other site was Home Oil(LSD 10-16-67-10 W5M). In upland boreal forested areas where rolling topography dominates, brine from a ruptured pipeline normally flows downslope on the surface of mineral soil, under the organic mat. Mosses that are at the bottom of hummocks and those at the bottom of the slope are subjected to a greater stress from the brine spill than those near the top of the hummock or slope. Field observations indicated an extremely variable pattern of damage to mosses (Innes and Webster 1980a).

Because of the variable pattern of damage to mosses in the field it was suggested that a study of moss/brine relations should be carried out under more controlled conditions. Moss carpeted the forest floor at two sites and the three main species were selected for greenhouse studies. Brine for this study was a sample from Home Oil Battery A3, near Swan Hills.

2.2 MOSSES

2.2.1 MOSSES AND EROSION

Moss biomass may be small but can represent an important portion of total production and nutrient cycling and should be considered in studies of ecosystem functions. Mosses contributed susbstantially to the system dynamics (Binkley and Graham 1981). Riely *et al.* (1979) stated that removal of bryophytes and humus would seriously reduce the amount of elements available annually for uptake by the trees and herbs.

In a study of *Hylocomium splendens* near a foundry emitting copper and zinc, Bengtson *et al.* (1982) made the following comments. "*Hylocomium splendens* is of considerable importance in the forest ecosystem. The moss covers much of the mor, thus regulating the microclimate near the ground. When the bryophyte cover is destroyed, large areas of land are laid bare with resulting changes in the microclimate. In the long run, soil organisms may suffer as a result of radical changes in composition of the litter and mor. Pollution by heavy metals prevents colonization by most other types of plants and the humus layer may eventually become subject to erosion" (Bengtson *et al.* 1982).

It is essential that soil and vegetation influences on erosion be considered together. Similar soil types often behave differently under different forest cover types (Singh 1983).

Forest floor serves a very important function in arresting erosion by:

1. absorbing the kinetic energy of falling raindrops (Metz and Farrier 1965; Wooldrige 1970)
2. absorbing and holding precipitation (Golding and Stanton 1972)
3. increasing infiltration and reducing evaporation from the soil (Moul and Buell 1955; Rowe 1955)
4. insulating soil surfaces from extremes in temperature (Price 1969; Wooldrige 1970; Armson 1977)
5. protecting bare soil from wind erosion (Richards 1929; Leach 1931; Kleiner and Harper 1972; Studlar 1980)

Physical soil properties of texture and structure, together with the nature and amount of the forest floor have a great effect on water infiltration, storage and movement. The forest floor contributes to the development of granular soil structure and increases biological activity, organic matter and non-capillary pore space in the soil (Arend 1941), all of which are conducive to high infiltration rates. Sheet erosion and associated gully erosion do not occur to any significant degree if the forest floor is intact (Kleiner and Harper 1972; Armson 1977).

The forest floor is an important part of the hydrologic system of a forested watershed. It has long been recognized that mosses, particularly Sphagna, play an important part in holding back the rainfall in forested regions, thus preventing floods and droughts (Grout 1912). The type and

thickness of the floor will determine the amount of water that can be held and the rate at which water can move into the mineral soil (Armson 1977). In a forest the impact of the rain is broken by various stories of branches and finally by forest floor cover. Thus water reaches the soil practically without kinetic energy (Kohnke and Bertrand 1959). Golding and Stanton (1972) found the water storage capacity of the forest floor was strongly related to the percentage of moss cover in the test area. Moss species varied in their capacity to hold water, and rates at which samples soaked up water and dried out, were different (Moul and Buell 1955). Assuming mosses dry out completely between storms, they may intercept as much as 50% of the throughfall where they occur. Lichens intercept about half as much as mosses. It seems probable that the densely packed upright plants of turf and cushion types may be the most efficient moss growth forms in the interception of moisture (Golding and Stanton 1972). Removal of bryophytes might cause a decrease in the water-holding capacity of the soil, and where the soil is shallow, erosion might eventually result (Rieley *et al*. 1979).

Aquatic mosses line the beds and banks of streams, which prevent erosion by the rushing water (Grout 1912). Emig (1918), Taylor (1919), and Richardson (1981) give examples of mosses playing a role in formation of rock-like tufa by deposition, on the plants, of calcium carbonate or iron from the water. Emig (1918) found *Didymodon tophaceus*

the most prevalent of three mosses distributed along moist ledges of shallow streams. The long branched moss stems fixed tangled masses of algae to the rock surfaces of the rapids or cascades. The water evaporates from the more exposed plant surfaces and calcium carbonate slowly crystallizes about the plant filaments. Young plants at the surface of the water continue to grow but the incased filaments gradually decay, leaving behind small calcareous tubules arranged like a mass of coarse fiber. The transition from moss plant to incrustation and finally to compact limestone is very slow. The type of travertine formed by the moss *Didymodon* depends largely on the original position of the growing plants. Taylor (1919) cited a similar occurrence of porous tufa formed from iron deposits on aquatic moss. It is also stated that floating islands originated from surface mats formed over the water. *Camplium stellatum* is one of the chief agents in the formation of surface mats.

There is a decided increase in the bryophytes and lichens after a fire (Moul and Buell 1955). The initial cover reduces splash erosion by raindrops and reduces temperature variation at the soil surface and improves moisture retention which leads to a more diversified community (Leach 1931; Moul and Buell 1955; Brown 1982). Grout (1912) cited an example of moss protonema protecting the soil from the terrific downpour of summer showers. Moss accumulated to depths of 25 cm or more, on tussocks at a Yukon test site. The moss provided excellent insulation

which helped to maintain the permafrost near the surface, and prevented solifluction, through the summer (Price 1969).

Marsh and Koerner (1972) described the role of moss in slope formation in coastal regions. "New microhabitats are created which lead to germination potentials not otherwise present. The moss lobe provides diversification of the slope environment. Moss is the dominant vegetative unit on these slopes because it readily tolerates sand burial. *Polytrichum piliferum* can appear from burial depths exceeding 6 cm and can regenerate 50% cover from burial up to 3.5 cm. The moss clumps reduce the ability of runoff to transport sand across it."

Leach (1931) listed *Polytrichum piliferum*, *Polytrichum juniperinum*, *Ceratodon purpureus* and *Rhacomitrium lanuginosum* as pioneers on sand and rock detritus. Species of *Polytrichum* differ from most other bryophytes in that they produce long underground stems, which turn brown and are very tough and strong. These moss species are able to grow for long periods in the absence of light. Leach found a community of *Polytrichum piliferum* going down to depths of 20 cm. In contrast, dead plants of the grass, *Festuca ovina* were found at various depths below the surface. *Polytrichum piliferum* and *P. juniperinum* are effective pioneers on sand because of their soil binding structures. *Rhacomitrium* grows over mountain top detritus and forms a protective cover against the elements but its rhizoids have no great soil binding effect, therefore patches of this species are

destroyed but the bare ground is stabilized by *P. alpinum*. *Ceratodon purpureus* is one of the pioneer species that grows on burn sites, however it may be eventually replaced by *P. juniperinum* and *Webera (Pohlia) nutans* if the site is covered by a layer of blown sand (Leach 1931).

Mosses play a very important role in dune colonization, by stabilizing the sand, adding organic matter and maintaining moist conditions near the sand surface through the water holding capacity of the gametophores and rhizoids. *Tortula ruraliformis* grows quickly through a one centimeter covering of sand. The leaves of this species, which have hair points, wrap around the stem in dry weather in a tight spiral. When dunes are dry, sand is blown about and the moss is likely to be covered. When rain moistens the sand, the mosses absorb water and leaves untwist so forcefully that sand grains are thrown to a distance of several centimeters. In this way each shoot digs a little pit in the sand cover (Richards 1929).

Kleiner and Harper (1972) compared two park sites and concluded that mosses and lichens may play a more important role in the stability of the highly erodable sandy soils of the grasslands than had been generally recognized. This cover provided protection during summer rains and heavy wind storms. *Tortula ruralis* and several species of lichens were instrumental in the buildup of organic matter and soil nutrients by cycling.

Studlar 1980, found that "trampling appeared to be more destructive to vascular plants in the *Polytrichum* plots, than to the moss itself. Regeneration of moss appeared to be most dramatic in those parts of the plots most severely damaged." Regeneration was greatest for *Polytrichum*, less for *Ditrichum* and the least for *Sphagnum*. It was concluded that, "trample-resistant structural features and high regenerative abilities apparently contribute to the importance of bryophytes on trails as potential inhibitors of soil erosion." Eroded areas could be revegetated using the method suggested by Bayfield (1976) of sowing a mixture of fragments from several species of mosses over bare areas.

2.2.2 MOSS: THE PLANT

"Variety upon variety, dark green and pale green; mosses like little fir trees, like plush, like malachite stars, like nothing on earth except moss."

Thomas Hardy

Mosses exist in all areas capable of supporting life (Huxley 1978). It is characteristic of mosses that they rarely occur as individuals but form extensive groups or colonies on moist soil, rocks and wood. In humid climates mosses may actually live on the leaves of higher plants. The largest mosses are native to the Southern Hemisphere and may exceed 30 cm in length, as does *Dawsonia*, but in North America the largest mosses, *Polytrichum* or haircap mosses, are usually no longer than 15 to 20 cm (Bold 1977).

"The individual moss plant consists of a slender leafy axis, either erect or prostrate, that may or may not have multicellular absortive structures called rhizoids" (Bold 1977). 'Rhizoids' are described as "simple or branched filaments, appearing dead at maturity, attaching the plant to the substrate, sometimes covering stems, rarely found at the back of costa or at leaf tips" (Crum and Anderson 1981). The role of rhizoids is not yet completely understood. The fact that they play a role in anchorage and in water conduction by capillary action is generally admitted. "The role in the active uptake of water and solutes has been less evidenced and published observations are not in complete agreement" (Hébant 1977). Mosses do not possess roots and except for those mosses with rhizome systems such as *Polytrichum*, *Dawsonia*, and *Climacium*, have very limited powers of withdrawing water from the substrate (Richardson 1981). Crum and Anderson (1981) described a 'rhizome' as "a slender, horizontal, subterranean rootlike stem." Wigglesworth (1947) described the rhizoid wicks of *Polytrichum commune*. It was stated "the rhizoid system of the Polytrichaceae, although highly specialized and important, is often neglected or confused with the 'rhizome' in descriptions of the species."

Any moss stem bearing a dense rhizoidal covering is well equipped for external capillary conduction of water. The significance of such rhizoids for water retention and absorption, is realized by considering the main strands are

direct outgrowths of superificial cells of the stem. Performing a similar function, but less widely distributed among mosses, are the paraphyllia. These cover the main stems of *Hylocomium splendens*, *Thuidium* species and some other mosses. Paraphyllia are minute, richly branched leaves of variable and irregular shape (Watson 1964).

"Individual moss shoots exert little control over water loss to dry air, while the cytoplasm itself may show a high degree of desiccation tolerance" (Proctor 1979). Bryophytes probably owe their success to at least two major adaptive features which must have surfaced early in their evolutionary history:

1. desiccation tolerance and
2. regeneration.

Mosses possess a type of protoplasm that can remain alive for long periods without water, even under conditions of high temperature. However, when resupplied with water through rain or dew they almost instantly (within 30 seconds in some species) resume photosynthesis (Dilks and Proctor 1979), if other factors such as temperature and light permit (Anderson 1980). This adaptation makes it very difficult to determine when a moss is 'dead'; therefore most bryologists prefer the term inactive. "Desiccation resistance in bryophytes is largely physiological and differences in morphology are of little importance" (Lee and Stewart 1971). Mosses vary in their desiccation tolerance depending on the time of year. Generally they are most sensitve during autumn

and early winter with their tolerance increasing through spring to a maximum in early summer (Richardson 1981). Desiccation tolerance varies greatly among species (During 1977) but generally correlates well with habitat (Proctor 1981).

Reproductive patterns enable mosses to circumvent some of the difficulties imposed by their extreme habitats. They persist because of their abilities to remain alive and regenerate when favorable conditions return. Almost all living parts of the moss: leaf, stem or rhizoid, as well as spores have the power of forming new plants (Garjeanne 1932; Watson 1964; Doyle 1970; Anderson 1980). In general, mosses produce vast numbers of spores. For example *Pleurozium schreberi* fruits abundantly in Canada and a carpet of the moss may produce 10^8 healthy spores/m^2 (Longton 1976). However, "the sexual cycle of moss is so dependent upon water and so vulnerable to the changes of weather during critical phases that vegetative persistence is extremely crucial" (Anderson 1980). Mosses have been reported "to exhibit more copious and varied means of vegetative reproduction than any other section of the plant kingdom" (Sachs and Vines 1882).

There are important differences between the physiology of mosses and vascular plants (Watson 1964; Proctor 1979). Compared with vascular plants, bryophytes represent an alternative strategy of adaptation to life on land. Surface/volume ratios of bryophytes are much greater than

those of vascular plants. For bryophytes, the micro environment is of primary importance, and the water conduction problems of these are much closer in scale to those of a single leaf of a vascular plant(Proctor 1982). "The small size and the absence of roots are indications that most bryophytes react in a different way to environmental stresses, such as short periods of drought and cold, in that they cannot draw upon moisture reserves in the substrate even for a short period" (During 1977). Mosses are limited by their characteristic mode of life but they are also liberated, as they can occupy hard substrates such as rocks and bark which are impenetrable to roots and so untenable to vascular plants. Bryophytes differ from vascular plants in that the gametophytes occupy the dominant phase of their cycle (Zamski and Trachtenberg 1976). The generation of moss which is most noticable is haploid, whereas in vascular plant it is diploid (Garjeanne 1932; Doyle 1970).

2.2.3 MOSS WATER RELATIONS

Bryophytes differ fundamentally from vascular plants in their water relations (Watson 1964). Several well established concepts concerning water economy in higher plants cannot be directly transposed to bryophytes. True stomata are absent from moss gametophytes but are present in spore capsules of almost all moss species. External conduction, more than internal conduction, plays a

fundamental role in water economy of many bryophytes (Hébant 1977).

Buch (1945; 1947) introduced terms to classify mosses on the basis of their water conductance, however there are various interpretations of these terms as different aspects are mentioned throughout his long papers. Buch mentioned the three terms and both Hébant and Bayfield extracted their definitions from material given in his two papers. Hébant 1977 cited three terms used by Buch as follows:

1. "*Endohydric forms* - those in which internal conduction plays the same role as in vascular plants.
2. *Ectohydric forms* - those whose water supply comes from external conduction coupled with water uptake over the whole surface of the gametophyte.
3. *Mixohydric forms* - those where both internal and external conduction are important."

Hébant commented that, "the majority of contemporary bryophyte gametophytes are either mixo-ectohydric or ectohydric, according to this terminology, and that truly mixohydric ones are not very abundant". He said, "strictly speaking, endohydric gametophytes apparently do not exist in bryophytes. Only the sporogonia of at least certain mosses are likely to have a truly endohydric habit."

Bayfield 1973 cited Buch 1947, using only two terms:

1. "*Endohydric types* - those in which there is a well differentiated internal water-conducting tissue and the presence of a perceptible cuticle.

2. *Ectohydric types* - those in which there is no internal conducting strand and a cuticle cannot be demonstrated."

Polytrichum is an example of an endohydric form and the feather mosses are examples of ectohydric forms. All bryophytes retain a need for external water conduction and much of the structure of bryophyte leaves is likely to be related to this requirement (Proctor 1979; 1981). External conduction takes place in capillary channels related to the form and arrangement of leaves. The wider the angle at which leaves stand out from the stem, the less will be this capillary conduction. Also, it will naturally tend to diminish with wider separation of the leaves along the stem (Watson 1964).

Water is a major factor controlling growth and distribution of mosses (Busby and Whitfield 1978). Growth form affects the capacity of a bryophyte shoot system for water storage, the distance which water must be conducted to the evaporating surface and the site and rate of water loss to the air (Proctor 1979). A correlation exists between growth forms and the water economy of the considered species (Brise 1957). Compact cushions are found in sites where periods of severe drought are to be endured, whereas some of the tallest forms are found only in almost constantly wet environments (Hébant 1977).

Since mosses have very limited powers of withdrawing water from the substrate, water required for metabolic processes is derived from water falling on or flowing over,

the plants. Additionally, water is required to transfer the sperm cells from antheridia to archegonia. These constraints have resulted in mosses:

1. "becoming aquatic,
2. being confined to continually moist habitats and
3. evolving the ability to be poikilohydric."

That is the plant dries almost as rapidly as its surrounding environment, but it can resume normal metabolic activity on remoistening (Richardson 1981).

Most mosses are poikilohydric and share the adaptation with lichens, many terrestrial algae, and such animals as tardigrades, nematodes and rotifers (Crowe 1971) but with few vascular plants (Richardson 1981).

"The metabolic differences between mosses and flowering plants is put in perspective by the fact that net photosynthesis in flowering plants decreases as soon as water potentials drop below -100 to -300 kPa (-1 to -3 bars). In contrast photosynthesis, in flowering plants, may reach zero or even become negative (e.g. respiration is exceeding fixation) when potentials drop below -1200 to -1500 kPa (-12 to -15 bars). Furthermore photosynthesis and respiration, in feather moss, continues until the water potential falls to about -5500 to -10^4 kPa (-55 to -100 bars) and does not resume until the plant is remoistened" (Busby and Whitfield 1978). Upon rehydration there usually is a burst of respiration at above normal rates, followed by gradual recovery in assimilation (Lee and Stewart 1971;

Bewley and Thorpe 1974; Peterson and Mayo 1975). The period of enhanced respiration observed in poikilohydric plants, following remoistening, enables repair of cell membranes and curtails cell leakage (Bewley 1979; Brown and Buck 1979)

Optimum water content for net photosynthesis varies widely among moss species. In most cases the rates of net photosynthesis rise to a well defined maximum, then decline often to 50% or less of the maximum value (Dilks and Proctor 1979; Longton 1980). "The optimal water contents are generally lower than the saturation values. The relationship between net assimilation rate and water potential is broadly similar in a variety of mosses. Water potential may provide a more useful index of the physiological state of the moss plant than does water content" (Busby and Whitfield 1978). Busby *et al.* (1978) concluded that "moss wet days", through the influence of water on photosynthesis is the most influential environmental parameter for growth of *Hylocomium*. Moss wet days were also considered important by Bates (1979). "The growth of four pleurocarpous mosses showed a close correlation with wetness, especially monthly rainfall minus potential evapotranspiration" (Pitkin 1975). In the High Arctic it appeared that the major factor in controlling growth and production of bryophytes was the amount of surface water present (Vitt and Pakarinen 1977).

2.2.4 FEATHER MOSSES

Hylocomium splendens, *Ptilium crista-castrensis* and *Pleurozium schreberi* are among those mosses termed "feather mosses" (Plate 1) and are the three prevalent moss species at the two Swan Hills experimental plots (Hettinger 1980). They are conspicuous components of vegetation types which occupy substantial areas in boreal regions of North America. Feather mosses form open carpets in better drained and usually shaded areas of upland forest (Busby and Whitfield 1978). Feather mosses are common to the spruce-fir, mixed coniferous and lodgepole pine forest types in the Swan Hills (Achuff and LaRoi 1977). All three are also present in the mixed wood forest but *Hylocomium* (Plate 2) is the most common species (Griffiths and Pharis 1976).

2.2.4.1 *Hylocomium splendens*(Hedw.)B.S.G.

Hypnum splendens Hedw., Sp. Musc., 1801, p.262
Hylocomium splendens(Hedw.)B.S.G. Bryol. Eur. 1852, Vol. 5, fasc. 45/51 (Crum and Anderson 1981).

Crum and Anderson (1981) listed five varieties of *Hylocomium* which apparently are a product of environmental rigors. They are: var. *alaskanum*(Lesq., and James) or (Lesq. and James)C. Jens., var. *alpinum* Kindb., var. *gracilius*, var. *obtusifolium*(Geh.)Par., and var. *tenue*.

'*Hylocomium*' means woods inhabiting. *Hylocomium* has a wide geographical distribution and occurs in a variety

of habitats in a number of vegetation types including alpine, subalpine, boreal and arctic. It grows on humus and soil in moist forests (Crum 1976). Lee and La Roi (1979) found *Hylocomium* was the most important, and most abundant, widespread bryophyte in Jasper National Park. It had a wider distribution, in terms of moisture regime and elevation than *Ptilium* or *Pleurozium*.

Crum (1976) described *Hylocomium* as follows: "Plants form dull, green or yellowish mats. Stems and branches are a reddish color." The stems are stiff and wiry, regularly two to three pinnate in horizontal fronds in a step-wise arrangement, each frond produced by an arched and ascending, stoloniform innovation arising from the middle of the previous year's growth." Crum (1976) referred to this species as 'Stair-Step Moss' because of the successive branching, which usually corresponds to annual increments or cycles of growth.

Each increment or segment is said to be initiated in autumn as a lateral bud of the previous segment (Bengtson *et al.* 1982). Stem elongation and branch production by the segment occurs mainly during late summer and autumn in the next year, and a bud representing a daughter segment is formed near the end of the growing season. However, little stem elongation in a parent segment follows initiation of its daughter segment (Longton 1980). Multiple segments more frequently arose from long than from short mother

segments with a frequency of 10 to 15% for twin segments. Branching is an important means of vegetative propagation in *Hylocomium* (Bengtson *et al.* 1982).

Busby (1976) reported that even when cyclic changes are observed in mosses, such patterns are not necessarily seasonal but reflect abrupt variations in temperature or water supply or both. Exposure to a very saline solution for three days is an abrupt physiological change for *Hylocomium* samples and regrowth, upon recovery, usually appears as a new segment (Ross Daley 1980). Poorly developed *Hylocomium* plants may be confused with *Pleurozium* however the presence of abundant paraphyllia (leaf-like scales produced on stems or branches of many pleurocarpus mosses) in *Hylocomium* is the best means of establishing the identity of doubtful specimens (Crum 1976).

Hylocomium is one of the most studied feather mosses because of its cyclic growth phenomenon. Annual growth increments enabled Tamm (1953) to examine its seasonal growth, yield and nutrition, and relate these to environmental factors such as light and rainfall. Bengtson *et al.* (1982) measured the weight, length and branching of the yearly segments to determine the effects of copper and zinc on its growth. Tamm claimed that the annual shoots of this moss are nutritionally isolated both from each other and from the soil. Soluble nutrients from leaves and bark of overstory are leached

out by rainwater, onto the moss carpet below, in quantities corresponding to the uptake by the moss. *Hylocomium* has an efficient absorbing system for the nutrients washed out of tree crowns (Tamm 1953). Of the three feather mosses, it is best adapted for external water transport because of the presence of paraphyllia. Water in the apical region tends to be held there unless the amount of available water is high enough to physically overflow the leaf axil into the one immediately below it (Busby 1976).

Tamm (1953) suggested that in *Hylocomium* potassium and sodium are accumulated in the young moss and then decreased in concentration, sodium very quickly, potassium at a slower rate as the moss matures. The bivalent and trivalent metals show a successive accumulation in the bud. Calcium concentrations increase with age on living and dead fronds. Calcium and potassium uptake is roughly of the same magnitude (Tamm 1953; 1964).

Tamm (1953) stated that moss growth is limited entirely, or partly by the nutrient supply or, beneath close canopy, by light. Beneath the tree canopy *Hylocomium* yield varies inversely with light intensity. At the same time nutrient concentration in the living moss increases with increases in shade, while the nutrient concentration of moss from outside the canopy was relatively constant, independent of exposure. The

individual variations in growth and nutrient concentrations were found to be very great in apparently homogeneous *Hylocomium* carpets (Tamm 1964).

Tamm (1964) observed the dependence of the *Hylocomium* carpet upon tree canopy. Outside the tree canopy, annual yield of moss decreased proportionally with the distance from tree crown projections until it was replaced by other species. Shrubs and heather may act as a substitute for trees. Exceptions from this dependence are found on steep rocky slopes, where water often trickles through luxurient *Hylocomium* tussocks. Steere *et al.* (1977) noted that in areas where trees had been cut down, a stunted physiological form of *Hylocomium splendens* occurred and it is often referred to as *Hylocomium alaskanum*.

Beil (1966) however used a multiple regression analysis of bryophyte cover in a subalpine spruce-fir forest and concluded development of the moss cover appeared to be independent of tree canopy. Busby *et al.* (1978) stated that, "the growth of *Hylocomium splendens* is so closely balanced with its microclimate that even the removal of a rather open shrub layer can have measureable effects on growth rates, however these effects are difficult to quantify since the growth of undisturbed moss is so variable."

PLATE 1: Feather mosses: *Hylocomium splendens* **(left),** *Ptilium crista-castrensis* **(center),** *Pleurozium schreberi* **(right). (graduations=1 cm)**

PLATE 2: *Hylocomium splendens*

2.2.4.2 *Ptilium crista-castrensis*(Hedw.)De Not.

Hypnum crista-castrensis Hedw., Sp. Musc. 1801 p.287
Ptilium crista-castrensis(Hedw.)De Not., Atti, R. Genova. (1869)1:101 (Crum and Anderson 1981)

Ptilium (Plate 3) has the most restricted geographical distribution and the wettest habitat of the three feather mosses (Achuff 1974; Achuff and La Roi 1977; Lee and La Roi 1979). Busby and Whitfield (1978) found it had the highest water content. It is a consistent member of the feather moss community but is rarely prominent (Busby *et al.* 1978). However it appears to be the most prominent feather moss in the Swan Hills Site 1 area. It is more prevalent on rotting logs at the Swan Hills site than the two other feather mosses (Ross Daley 1980).

Ptilium was dominant in the oldest *Picea-Abies* forests in the Swan Hills. The *Rubus/Ptilium* community was associated with higher altitude, higher precipitation, cooler summer temperatures, and lower soil nutrient availability than the *Viburnum/Hylocomium* community (Achuff 1974; Achuff and La Roi 1977). *Ptilium* tends to occur on steep, moist slopes of calcareous soil (Steere 1979). In a white spruce forest in Central Alberta, Winner and Bewley (1978) found that *Ptilium* and *Hylocomium* initially increase in number when the more sulfur dioxide sensitive and dominant *Pleurozium* diminished importance along a stress gradient.

"*Ptilium* plants are strikingly handsome and grow in shiny mats that are bright-green, yellow or golden in color. The feathery fronds curved at the tips are appropriately called the Knight's Plume. 'Crista' means a crest and 'castrensis' means castle or fort" (Crum 1976). It is reported to sporulate very rarely, but where sporophytes are produced they can be rather abundant (two to five per frond)(During 1979). *Ptilium* was a 'must' for Victorian moss collectors because of its beauty; however it has received the least amount of attention of the three feather moss species in modern day experiments.

PLATE 3: *Ptilium crista-castrensis*

2.2.4.3 *Pleurozium schreberi*(Brid.)Mitt.

Hypnum schreberi Brid., Musc. Recent., (1801)2(2)88

Hylocomium schreberi(Brid.)De Not., Cron. Briol. Ital. (1867)12:15

Calliergonella schreberi(Brid.)Grout, Moss. Fl. North Amer. (1931)3:103

Pleurozium schreberi(Brid.)Mitt., J. Linn. Soc. London Bot. (1869)12:537 (Crum and Anderson 1981)

Pleurozium (Plate 4) has received more attention from researchers than *Ptilium* and although ecologically similar to *Hylocomium*, *Pleurozium* has a more restricted geographical range in North America. It occurs mainly in boreal and subarctic regions (Busby *et al.* 1978) where it is found on humus, soil and other substrates, usually in dry, open woods, but also in bogs or wet coniferous woods. Plants occur in loose, pale green to yellow mats (Crum 1976).

Pleurozium causes confusion today, not because of its various names but because of its various physiological forms of expression throughout the world. Data indicate *Pleurozium* has either plastic or genetic adaptation to different environments. It is at least functionally dioecious and no consistent differences in growth rate between male and female plants have been found. Functional male plants have been found to be widespread in northern Scotland and southern Scandinavia, but rarely farther south, over much of

southern Britain and parts of France (Longton and Greene 1969). Rate of growth increased with increase in temperature, independently of photoperiod, over the range 5 to 20°C. The temperature optima for net assimilation varied depending on the environment of the samples (Longton and Greene 1979).

Crum (1976) of Michigan, in another example, reported "widespread occurrence of *Pleurozium* in the Northern Hemisphere, and its disjunction in Central and South America are difficult to understand, as it seldom fruits and has no mode of vegetative production." Contrary to Crum's findings, samples of *Pleurozium* collected at Swan Hills and Villeneuve had an abundance of spore capsules.

The vegetative method of reproduction in *Pleurozium* was investigated by Longton and Greene (1979) in a series of experiments on leaf generation. "In mature gametophores of *Pleurozium* the stems are predominantly red in color, but a green apical region, where the internodes are expanded and the red pigmentation has not developed, extends up to 5 mm behind the terminal bud during the growing season. The capacity for regeneration was found to be restricted largely to leaves from the uppermost green branches and from the green apical region of the subsequent branches. Regeneration was not observed in leaves taken from branches which had attained their mature red color throughout." Bates

(1979) stated that "no absolute demarcation can be drawn between 'dead' and 'living' shoot segments as different physiological processes decline at different rates as aging occurs."

Crum (1976) cited Uggla's 1958 findings that *Pleurozium* and *Hylocomium* often lack connections with the substrates and are therefore usually completely consumed by forest fires. For example, in Northern Finland these species colonize burns only after thirty to forty years. This lack of direct contact with the substrate has made it useful in air pollution studies (Rinne and Barclay-Estrup 1980). Tests by Longton and Greene (1979) showed that the addition of a nutrient solution to the soil around the base of the plants proved ineffective but spraying it directly onto the leaves prevented the occurrence of deficiency symptoms.

Achuff (1974), Achuff and La Roi (1977), and Lee and La Roi (1979) group *Pleurozium* and *Ptilium* together in terms of their moisture regime. Both have similar substratum affinities (e.g. percentage occurrence on wood and humus)(Lee and La Roi 1979). The three feather mosses are the dominant mosses in the upland areas of coniferous forests in Alberta (Brinkman 1929; Busby 1976; Achuff and La Roi 1977; Lee and La Roi 1979; Hettinger 1980).

PLATE 4: *Pleurozium schreberi*

2.2.5 MOSSES AND SALTS

Brown (1982) presented an overall view of moss/salt relations. Proctor (1981;1982) addressed the topics of mineral nutrition and excess salts in moss with two presentations on bryophyte physiology. Tamm (1953; 1964) reported his classical study of nutrition in *Hylocomium splendens* carpets. Longton and Greene (1969; 1979) have studied nutrition and reproduction in *Pleurozium schreberi*. Rinne and Barclay-Estrup (1980) used *Pleurozium schreberi* to study heavy metal pollution in Canada. Rühling and Tyler (1970), and Bengtson *et al.* (1982) used *Hylocomium splendens* to study heavy metal pollution in Scandinavia. Shacklette (1965) stated that certain species of bryophytes are known to be either commonly or exclusively associated with mineral deposits, e.g. 'Copper mosses'. However Brown (1982) suggested a single explanation for the occurrence of all species of 'Copper mosses' is very unlikely.

The salts of sodium, potassium, calcium, and magnesium are of particular interest in the present study. Shacklette (1965) stated that bryophytes are generally very intolerant of salt although some species can endure relatively large amounts. Moss species in Utah will tolerate up to about 0.5% sodium chloride. In Alaska, Shacklette found six bryophyte species on substrates that were submerged in sea water at high tide and twenty three species at sites subjected to sea spray. *Grimmia maritima* grows on coastal rocks coated with sea salt.

In 1978, Lewis Smith concluded that, unrelated to the distribution of individual species, the sodium concentration in bryophytes:

1. "decreased with horizontal and vertical distance from the coast
2. increased, up to five-fold, in the summer
3. increased with wetness of the habitat and increased plant water content."

Potassium concentrations were higher than those of sodium, but summer potassium levels were frequently less than those in winter. The concentration of calcium varied considerably and showed little consistency in seasonal fluctuation.

Bates and Brown (1974) compared three seashore mosses (*Grimmia maritima*, *Tortella flavovirens* and *Ulota phyllantha*) to four inland species (*Grimmia pulvinata*, *Grimmia trichophylla*, *Tortella tortuosa* and *Ptychomitrium polyphyllum*) using artificial seawater solutions. Potassium loss from inland mosses occurred in seawater concentrations exceeding 50% whereas seashore species withstood concentrations of artificial seawater up to 200% with little change. Treatment of the seashore moss, *Grimmia maritima*, with sodium chloride at the concentration in seawater produced almost complete loss of intracellular potassium. This effect was greatly alleviated by calcium, partially by magnesium and was nullified when sodium was accompanied by both calcium and potassium. *G. pulvinata* (inland moss) suffered potassium loss when immersed in seawater even when

the external calcium concentration was increased five-fold. Maritime species were not permanently damaged by immersion in seawater, but inland species were permanently affected (Bates 1976). Photosynthesis, protein synthesis, and chlorophyll concentrations were severely decreased in inland mosses compared to maritime mosses when treated with artificial seawater. Loss of potassium also occurred when *Fontinalis antipyretica* was treated with mannitol solutions having osmotic potentials equal to that of seawater (Brown 1982).

Magnesium was the prevalent cation in an alkaline industrial waste colonized by bryophytes in Britain. A *Barbula-Funaria* community grew on soils with a Mg/K ratio of 52/1 and a more flushed community (near a stream) of *Puccinellia* and *Pohlia proligera* had a Mg/K ratio of 11/1. There is a wide variation in the absorptive capacity of different moss species for the four cations sodium, potassium, calcium and magnesium, which varies with ecological habitat (Mirza and Shimwell 1977).

Boerner and Foreman (1975) compared the effects of salt spray on *Ceratodon purpureus*, *Aulacomnium palustre* and *Polytrichum commune* from three different habitats. Their three treatments were:

1. "simulated salt spray alone, for thirty three of forty four days
2. salt spray for thirty three of forty four days plus five simulated rains

3. control, fresh water misting for thirty three of forty four days."

Following treatment applications a fourteen day recovery period followed, during which plants were misted with fresh water on all fourteen days. Percent green tips, opposed to brown tips, was the parameter of measurement.

Boerner and Foreman (1975) found no significant differences in samples from the different habitats. It was concluded salt tolerance of moss is a species level trait, not a population level trait. There was also no direct relationship between soil type and salt tolerance of the various species. None of the three species survived the salt spray alone. *Ceratodon* showed some tolerance to salt spray when periodic rain was included. Boerner and Forman placed the three moss species in two groups used by Martin (1959) to classify the salt tolerance of vascular plants. However this grouping should be viewed with some caution considering the difference in the physiology of vascular plants versus mosses, especially the regeneration capabilities of mosses.

Most moss species found in saltmarshes are widespread and common in a range of inland habitats, with *Pottia heimii* more frequent in saltmarsh sites than elsewhere in Britain (Adam 1976). The lowest stands with abundant representation of mosses are flooded by at least one hundred tides a year and included *Pottia heimii*, *Amblystegium serpens*, *Eurphynchium praelongum*, *Cratoneuron filicinum*, *Campylium polygamum* and *Grimmia maritima*. The majority of species in

Adam's list are found in areas liable to experience between twenty and fifty flooding tides in a year. There is considerable variation in the moss assemblages found on different marshes in various European countries, e.g. species present, dominant species, growth forms, numbers and substrate.

Vegetation changes occurring during land reclamation from the sea was studied by Joenje and During (1977) who observed initial colonization by 'weedy' bryophyte species such as *Funaria hygrometrica*, *Marchantia polymorpha*, and *Physcomitrium pyriforme* in areas where salt concentrations were rapidly reduced. In coarse sandy areas, *Funaria* grew in the wet winter months but was killed during the summer when desiccation caused increased salt contents at the soil surface. In silty sands, salinity declined slowly and the first colonist was *Pottia heimii*.

For three years Rowell and Crepin (1978) monitored the natural recovery of a very wet, Black Spruce/*Sphagnum* Bog near Swan Hills that was the site of a salt water spill. The time of the spill was not known, however they speculated it was two or three years before monitoring began. Considerable damage to the vegetation had occurred as a direct result of the high salt concentration. Natural leaching by rainfall reduced the concentration of salt at the soil surface to levels that allowed most of the affected area to revegetate, including the moss hummocks.

Hettinger (1980; 1983) monitored the upland vegetation at the brine spill reclamation plots in the Swan Hills. Main treatments were control, brine plus flush, brine plus flush plus gypsum, and brine only. The three subtreatments included no seed-no fertilizer, seed plus fertilizer, and fertilizer. Having no roots may be an advantage for most upland mosses in a spill situation because there is no prolonged contact with saline soil. Species such as *Sphagnum* which occur in wet, poorly drained areas may be severely damaged by salt spills because brine tends to pool in depressions and at the bottom of slopes. Theoretically the exposure period to brine should be shortest for most bryophytes and lichens, followed by shallow rooted vascular plants and then by rooted woody species.

The upland mosses, *Hylocomium splendens*, *Ptilium crista-castrensis*, and *Pleurozium schreberi* were monitored by Hettinger over a four year period. *Hylocomium* showed a high proportion of severely damaged tissue, although less extreme than for balsam fir seedlings. This moss exhibited a high mortality in the first year, especially in depressions where brine accumulated during application, and recovered relatively slowly at both test sites. It was concluded *Hylocomium* has a greater sensitivity to salinity, and recovery is slower than for the other feather mosses.

Ptilium exhibited damage, but to a lesser extent than *Hylocomium*. Atypical of the general response trends of other plants, *Ptilium* often had the lowest proportions of damaged

tissue in the brine only plots. *Pleurozium* showed a more variable response but was most severely damaged by the brine only treatments, however it exhibited a marked recovery by the end of the second growing season. *Pleurozium* seemed more resilient than *Hylocomium* and was considered more salt tolerant. The pattern of damage to mosses was highly variable because those at the base of hummocks made contact with the brine whereas those at the top of the mounds frequently escaped. For this reason Hettinger (1983) considered mosses difficult to use as monitoring species in field experiments.

Difficulty in monitoring moss response was also experienced in preliminary laboratory studies (Ross Daley 1980) that preceded the present study. After a three day contact period with brine solutions, most of the mosses turned brown, however after a recovery period in the greenhouse, green regrowth appeared. Of the methods tested, linear measurement of regrowth was the most satisfactory indicator of plant response. *Pleurozium schreberi* was the reference species in experiments that also included *Hylocomium splendens*, *Ptilium crista-castrensis*, *Dicranum polysetum* and *Polytrichum commune* (Daley Ross 1980). The mosses' Salt Shock Tolerance was tested because plants were exposed, for a short period of time, to a brine with a high salt content. Salt content was measured by electrical conductivity. This differs from Salt Tolerance, where a plant grows at a relatively constant salt content or

electrical conductivity throughout its life (Ross and Webster 1983). Plants were in contact with brine solutions (0 to 60 mS/cm) for three days, flushed with distilled water and allowed to recover in the greenhouse. Regrowth of most species decreased suddenly in the 20 to 30 mS/cm treatment range. *Hylocomium* regrowth decreased at a lower electrical conductivity level than most other species and was considered the most salt sensitive species. *Pleurozium* was considered the most salt tolerant species and the response of *Ptilium* was between that of *Hylocomium* and *Pleurozium* (Daley Ross 1980).

The most beneficial spill treatment tested was the water flush. A calcium nitrate solution (0.07 M) reduced regrowth in all three feather mosses, whereas *Pleurozium* showed the best response to a gypsum treatment. Response of *Ptilium* to water flush and gypsum solutions was similar while regrowth was reduced somewhat in the fertilizer treatment. *Hylocomium* regrowth in gypsum and fertilizer treatments was about 65% and 50% respectively, of that obtained in water flush treatments (Daley Ross 1980).

In addition to using regrowth to measure response some of other methodology findings were, the importance of high humidity to prevent salt deposits on moss tips during the treatment phase and to increase the amount of regrowth during the recovery phase. Moss segments, wrapped with nylon net, inserted into plastic vials was the favored method for applying treatments because the amount of space and

solutions could be reduced. Bundles were closer to the moss spacing in nature. All treatments were applied as solutions and bundles were immersed in a 1/5 nutrient solution during recovery (Ross Daley 1980).

There was an indication that moss response to non-salt, osmotic stress (mannitol) varied from response to osmotic plus specific ion stress (brine) (Ross 1981, unpublished results). Slayter (1967) stated that progressive reductions in growth rate caused by increased salinity appeared to be caused primarily by the effect of excess ion accumulation in the affected plants. Direct osmotic effects, acting through reduced water availability to the plants appear to be of secondary importance. Salinity effects on plants have a dual nature in that salts change both water relations and the electrolyte balance of plant tissues. If there is no salt uptake but low water potentials as in non-electrolytic, osmotic solutions of mannitol or sorbitol, the consequences will be the same as for plants grown under drought stress (Greenway 1973).

In a review of osmoregulation in plants, Hellebust (1976) commented "little is known about moss osmoregulatory functions." Within a publication by Lange *et al.* 1983, Wyn Jones and Gorham (1983) give an updated reveiw of osmoregulation in plants however, nothing is said about moss. 'Moss' or 'bryophyte' does not appear in the index of this publication.

3. METHODS AND MATERIALS

3.1 SPECIES AND SOURCES

The three prominent moss species at the Swan Hills reclamation plots (*Hylocomium splendens*, *Ptilium crista-castrensis* and *Pleurozium schreberi*) were used in Experiments 1, 3 to 6. *Ptilium* and *Pleurozium* were used in Experiment 2. Moss samples came from the Swan Hills or Villeneuve areas of Alberta. They were either put directly in the cooler (wet storage) or air dried, then put in the cooler (dry storage). The wet storage samples were gathered in the area of the Swan Hills reclamation plots on July 9, 1980. Mosses, especially *Hylocomium*, turned brown when held in the cooler in a wet condition therefore dry storage was adopted. The Villeneuve samples were gathered on May 5, 1981 and Swan Hills samples on June 4, 1981.

Brown moss refers to segments that were more brown than green, actually the rejects of material stored by the wet storage method. The brown *Hylocomium* segments had already been discarded therefore it was absent in Experiment 2. With the original starting material being brown colored, any regrowth would appear as green colored material and therefore the two could be easily separated.

Samples were best sorted in a dry or slightly damp state. Moss segments or tips (top 20 mm) were selected, trimmed, washed in tap water to remove debris, rinsed in distilled water and excess moisture removed by centrifugal

force. Each replicate contained twenty five individual moss segments or tips and each treatment was replicated six times in randomized block or factorial designs.

3.2 EXPERIMENTAL PROCEDURES

All six experiments followed three main procedures (Table 3). Two types of culture (BAG or NET) were used and two methods of regrowth measurement were tested (Bag Weight Difference and Clipping Weight). Stress solutions (Distilled Water, Brine or Sorbitol) were applied for seventy two hours (three days) in Experiments 1 to 4 and 6. In Experiment 5 the stress was applied for two, four, six, eight or ten days.

TABLE 3: Outline of procedures used in the six experiments

EXPERIMENTS 1+3	*EXPERIMENTS 2+4+5*	*EXPERIMENT 6*
WEIGH		
STRESS	STRESS	STRESS
FLUSH	FLUSH	FLUSH
DRY		
WEIGH		POST FLUSH
RECOVERY	RECOVERY	RECOVERY
DRY	DRY	DRY
WEIGH		
CLIP	CLIP	CLIP
WEIGH REGROWTH	WEIGH REGROWTH	WEIGH REGROWTH

3.3 BAG CULTURE

Bag and Net Cultures involved different plant lengths, different containers, different plant positions for recovery, as well as different weighing methods. I designed and manufactured all of the bags and bundles used throughout the experiments. In 'Bag Culture' (Experiments 1 and 3) twenty five moss tips were added to a polyester bag (approximately 5 X 10 cm) made of sheer polyester curtain material. There was an indication that some moss particles were lost when bags and moss were immersed in a liquid. Therefore weights were recorded before and after the stress was applied. The weight of bag plus moss was recorded at 80% relative humidity. Each bag was then placed in a vial, positioned in one cell (4 X 6 cm) of a six celled plastic propagation insert. A large plastic tray (26 X 52 cm) held eight of the six celled inserts (Model 806, WestCan Horticultural Supplies) and a total of forty eight vials. Amber colored vials (12 dram size, 65 ml) were used in an attempt to reduce light and surpress growth of algae in the nutrient solution. Both tray and inserts had drainage holes.

The stress solutions were added to the vials for seventy two hours (contact time). Plants were drained, then put in a distilled water flush for one hour (the six replicates were placed in a one liter container of distilled water). Polyester bags plus moss were air dried and stored at 80% relative humidity then weighed. Bags and plants were then placed in a vertical position in clean, dry vials for

recovery in the greenhouse tunnel. After the recovery period, polyester bags and moss were dried and weighed at 20% relative humidity. The change, before and after recovery, (Bag Weight Difference) was calculated for 20% relative humidity with a conversion factor derived by a standard set of polyester bags plus moss weighed at both 20 and 80% relative humidity. It was speculated that the Bag Weight Difference, used only in Bag Culture, could be subject to a number of extraneous losses and gains in the system, therefore after the Weight Difference was recorded, regrowth was clipped from the original plants. Clippings were put in small pellon bags (approximately 3 X 7 cm) to weigh regrowth at 20% relative humidity and the Clipping Weight (Plate5) calculated. The Clipping Weight was the only method of weighing used in Net Culture, described in the next section. The Bag Weight Difference and Clipping Weight are compared in Experiments 1 and 3.

3.4 NET CULTURE

In Net Culture (Plate 6)(Experiments 2, 4, 5 and 6) twenty five moss segments were wrapped with a nylon net disk and tied with thread to form a cone shaped bundle. The net bundle was put in a vial, positioned in the plastic insert cell and the stress applied for the given amount of time followed by the distilled water flush. In Experiment 6 a factorial design was used for post flush treatments with seven levels of calcium and three levels of potassium. After

the flush, net bundles were left to drain overnight before post flush treatments were applied for seventy two hours. Bundles were then drained and placed in a horizontal position, on the bottoms of clean plastic cells for recovery. Regrowth was then 90° to the original material. Plants in Experiments 2, 4, and 5 were treated in a similar manner but went directly to the greenhouse for recovery after the distilled water flush. After recovery, samples were air dried, the regrowth clipped, and put into small pellon bags. Pellon bags plus moss were held at 20% relative humidity to weigh regrowth and the Clipping Weight calculated by subtracting the weight of pellon bag alone at 20% relative humidity.

A large magnifying glass mounted on a stand, forceps and a dissecting microscope light were used in the removal of regrowth from the original material (Plate 5). Adequate lighting was necessary to prevent eye strain. A Type H6 Mettler Analytical Balance was used for weighings and the standard set of polyester bags plus moss, was weighed prior to each weighing session.

PLATE 5: Regrowth removed and weighed in pellon bags for Clipping Weight

PLATE 6: Net Culture; Expts. 2, 4, 5 and 6

3.5 SOLUTIONS

All treatments were in solution form and were applied in the laboratory, plants were then moved to the greenhouse for recovery. Vials included snap on caps which helped hasten operations, especially in Experiment 6. Solutions were prepared ahead of time, added to the vials and capped until plants could be inserted. Distilled water only was the 'stress' (Table 3) applied for seventy two hours in Experiment 1. Brine and sorbitol were used in Experiments 2, 3 and 4 and brine only in Experiments 5 and 6. Both brine and sorbitol were adjusted to pH 7 with sodium hydroxide and hydrochloric acid. The brine (Table 4) was collected from Home Oil Battery A3, near Swan Hills. The sorbitol solutions, which gave the osmotic effect without the specific ion effect, were made to the electrical conductivity equivalent of the brine solutions using the osmosity[1] of mannitol (Weast 1979). Mannitol has the same empirical formula and molecular weight as sorbitol, but different positions for the hydroxal (-OH) and hydrogen (-H) groups.

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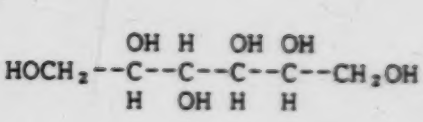
[1]Molar concentration of NaCl with same freezing point or osmotic pressure as given solution

d-SORBITOL



d-MANNITOL

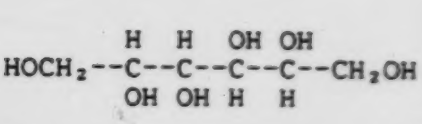


FIGURE 3: Structure of sorbitol and mannitol

Five levels or concentrations of brine and sorbitol (0 to 40 mS/cm) were compared in Experiments 2, 3, and 4 with a contact time of seventy two hours (three days). Four concentrations of brine only (0 to 60 mS/cm) were used in Experiment 5 with contact times from two to ten days. Experiment 6 used brine at 30 mS/cm applied for seventy two hours, followed by distilled water flush. Post Flush Treatments with seven levels of calcium sulfate (0 to 300 mg/L(ppm)), three levels potassium sulfate(0 to 100 mg/L(ppm)) or combinations of the two, for a total of twenty one different treatments, were added to the vials for seventy two hours.

TABLE 4: Analysis[3] of brine sample Home Oil Battery A3, Swan Hills

Chloride	27,030	mg/L(ppm)
Sodium	14,457	"
Calcium and Magnesium	3,026	"
Sulfate	634	"
Bicarbonate	286	"
Potassium	205	"
Ammonium-nitrogen	44	"
Nitrate-nitrogen	<1	"
Boron	23.7	"
Iron	1.9	"
Phosphorus	0.1	"
pH	7.1	
Electrical Conductivity	67.7	mS/cm
Sodium Adsorption Ratio	92.4	

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[3] Alberta Environment, Earth Sciences Division Lethbridge, Alberta

3.6 RECOVERY

Longton and Greene (1979) found if the nutrient solution was applied directly onto moss leaves, instead of to the growing media, nutrient deficiencies were prevented. Therefore unlike my preliminary experiments, mosses were misted with nutrient solution rather than being immersed in it. The macro nutrient solution was an adaptation from Basile (1975). The cobalt concentration was used by Hewitt (1952) and the rest of the micronutrients was an adaptation from Cooper (1975). A 1/20 nutrient solution (Table 5), made with distilled water, was misted on the plants during recovery in the 'tunnel' in the greenhouse. The 'tunnel' consisted of 6 mil plastic draped over metal arches, which were attached to a greenhouse bench. Containers of water and a film of water on the plastic on the floor of the bench were used to raise the relative humidity. The greenhouse temperature was 20°C±2 with a photoperiod of eighteen hours. Cool white fluorescent light was used to supplement natural light. A double layer of cheese cloth was placed on the south side of the tunnel to shade the moss.

TABLE 5: Full strength (1/1) stock solution for 1/20 nutrient solution misted on mosses during recovery

Chemical	g/liter	mg/L(ppm)	mg/L(ppm)
$Ca(NO_3)_2.4H_2O$	1.00	170.0 Ca	119.0 NO_3-N
$(NH_4)_2SO_4$	0.50	106.0 NH_4-N	363.0 SO_4
$Na_2HPO_4.H_2O$	0.05	14.4 Na	9.7 P
$MgSO_4.7H_2O$	0.25	24.6 Mg	97.4 SO_4
KH_2PO_4	0.20	57.5 K	45.5 P
Chelated iron[1]	0.01	1.1 Fe	
Micronutrients[2]	1.0 ml	BELOW	

[1]Na ferric diethylenetriamine pentaacetate
[2]Micronutrient formula given in Table 6

TABLE 6: Micronutrient stock solution used in (1/1) stock solution (1 mL/liter of full strength stock solution)

Chemical	g/liter	mg/L(ppm) (In 1/1 solution)	
$MnSO_4.H_2O$	1.5152	0.50	Mn
H_3BO_3	1.7162	0.30	B
$CuSO_4.5H_2O$	0.2750	0.07	Cu
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0129	0.007	Mo
$ZnSO_4.7H_2O$	0.3080	0.07	Zn
$CoCl_2.6H_2O$	0.0010	0.00025	Co

3.7 RELATIVE HUMIDITY

From the literature and from experience in my previous experiments, it was concluded that relative humidity was a factor to consider during the experiments. A plastic covered the plants during the treatment stage in the laboratory, to prevent salt or sorbitol deposits on moss tips. Water was added to the tunnel to help keep the relative humidity higher than the open air in the greenhouse.

It was speculated that the amount of regrowth produced by the moss would be very small and therefore changes in laboratory relative humidity from day to day may influence the sample weights. To reduce the variations somewhat, two holding cabinets one for high relative humidity and the other for low relative humidity were constructed with wooden frames (52 X 52 X 52 cm), covered with 6 mil polyethylene film. The cabinets were used to hold the bundles and polyester bags before treatment (80%) and polyester and pellon bags (20%) before weighing. The exception to this was in Experiments 1 and 3 where polyester bags were held at 80% relative humidity in the 'before' recovery weighing (see Table 3).

Two saturated salt solutions were used to control relative humidity (Winston and Bates 1960). Petri dishes of each solution were placed in the bottom of their respective cabinet and a protective plastic screen placed over top to prevent moss samples from falling into the solutions. Saturated potassium acetate gave about 20% relative humidity

and saturated ammonium sulfate, gave about 80% relative humidity. Water was added or removed from the petri dishes of salt solutions over time as the relative humidity of the laboratory varied. Potassium acetate takes a long time to reach its saturated state, when making a new solution (Winston and Bates 1960). Therefore instead of renewing the solution, periodically all petri dishes were emptied into a large beaker and the water boiled off. Any extra was stored in a plastic container and added to the dishes when necessary.

During the weighing process, plastic trays of sample bags were put inside horizontally placed double plastic bags. This was to minimize the change in weight caused by variation in relative humidity between the holding cabinets and the balance. One sample bag at a time was transferred to the balance and then back into an empty tray inside the plastic bags. The tunnel and holding cabinets were designed by the researcher. A summary of the experiments follows.

3.8 SUMMARY OF EXPERIMENTS

EXPT. 1: WEIGHT vs TIME (Green Tips, Bag Culture)

SPECIES (Spp.): *Hylocomium, Ptilium* and *Pleurozium*

MOSS SOURCE: Villeneuve; May 5, 1981; dry storage

STRESS AGENT: Distilled Water **LEVEL:** E.C.' 0 mS/cm

CONTACT TIME: 72 hours (3 days); June 2-5, 1981

RECOVERY TIMES:

4	weeks	June 5-July 15, 1981
7	weeks	June 5-Aug. 5
10	weeks	June 5-Aug. 26
13	weeks	June 5-Sept. 16
16	weeks	June 5-Oct. 7
19	weeks	June 5-Oct. 28
22	weeks	June 5-Nov. 18
25	weeks	June 5-Dec. 9

OBSERVATIONS: 3 Spp. X 8 Times X 6 Reps.= 144

MEASUREMENTS: Bag Weight Difference; Clipping Weight

EXPT. 2: BRINE/SORBITOL (Brown Segments, Net Culture)

SPECIES (Spp.): *Ptilium* and *Pleurozium*

MOSS SOURCE: Swan Hills; July 9, 1980; wet storage

STRESS AGENTS: Brine or Sorbitol

LEVELS: E.C. equivalents 0, 10, 20, 30, 40 mS/cm

CONTACT TIME: 72 hours (3 days); June 8-11, 1981

RECOVERY TIME: 17 weeks; June 11-Oct. 7, 1981

OBSERVATIONS: 2 Spp. X 2 Stress X 5 Levels X 6 Reps.= 120

MEASUREMENTS: Clipping Weight

'E.C.=Electrical Conductivity

EXPT. 3: BRINE/SORBITOL (Green Tips, Bag Culture)

SPECIES (Spp.): *Hylocomium, Ptilium* and *Pleurozium*
MOSS SOURCE: Swan Hills; July 9, 1980; wet storage
STRESS AGENTS: Brine or Sorbitol
LEVELS: E.C.' equivalents 0, 10, 20, 30, 40 mS/cm
CONTACT TIME: 72 hours (3 days); June 7-10, 1981
RECOVERY TIME: 22 weeks; June 10-Nov. 10, 1981
OBSERVATIONS: 3 Spp. X 2 Stress X 5 Levels X 6 Reps.= 180
MEASUREMENTS: Bag Weight Difference; Clipping Weight

EXPT. 4: BRINE/SORBITOL (Green Segments, Net Culture)

SPECIES (Spp.): *Hylocomium, Ptilium* and *Pleurozium*
MOSS SOURCE: Villeneuve; May 5, 1981; dry storage
STRESS AGENTS: Brine or Sorbitol
LEVELS: E.C. equivalents 0, 10, 20, 30, 40 mS/cm
CONTACT TIME: 72 hours (3 days); Aug. 7-10, 1981
RECOVERY TIME: 30 weeks; Aug. 10, 1981 to Mar. 10, 1982
OBSERVATIONS: 3 Spp. X 2 Stress X 5 Levels X 6 Reps.= 180
MEASUREMENTS: Clipping Weight

'E.C.=Electrical Conductivity

EXPT. 5: BRINE-EXPOSURE TIME vs LEVEL
(Green Segments, Net Culture)

SPECIES (Spp.): *Hylocomium, Ptilium* and *Pleurozium*

MOSS SOURCE: Villeneuve; May 5, 1981; dry storage

STRESS AGENT: Brine

LEVELS: E.C.' 0, 20, 40, 60 mS/cm.

CONTACT TIMES:

48 hours	(2 days)	Sept. 27-Sept. 29, 1981
96 hours	(4 days)	Sept. 27-Oct. 1
144 hours	(6 days)	Sept. 27-Oct. 3
192 hours	(8 days)	Sept. 27-Oct. 5
240 hours	(10 days)	Sept. 27-Oct. 7

RECOVERY TIME: 30 weeks; Oct. 7, 1981-May 3, 1982

OBSERVATIONS: 3 Spp. X 4 Levels X 5 Times X 6 Reps.=360

MEASUREMENTS: Clipping Weight

'E.C.=Electrical Conductivity

EXPT. 6: Ca/K[1] POST FLUSH TREATMENTS
(Green Segments, Net Culture)

SPECIES (Spp.): *Hylocomium*, *Ptilium*, and *Pleurozium*

MOSS SOURCE: *Hylocomium*;
Swan Hills; June 4, 1981; dry storage
Ptilium and *Pleurozium*;
Villeneuve; May 5, 1981; dry storage

STRESS AGENT: Brine

LEVEL: E.C.[2] 30 mS/cm.

CONTACT TIME: Brine for 72 hours (3 days); Dec. 5-8, 1981

POST FLUSH TREATMENTS: 21 treatments (7 Ca X 3 K)

CONTACT TIME: Ca/K for 72 hours (3 days); Dec. 9-12, 1981

$CaSO_4.2H_2O$

FACTORIAL	0	50	100	150	200	250	300 mg/L (ppm) Ca
K_2SO_4	50						
	100						

mg/L (ppm) K

RECOVERY TIME: 25 weeks; Dec. 12, 1981 to June 7, 1982

OBSERVATIONS: 3 Spp. X 7 Ca X 3 K X 6 Reps.=378

MEASUREMENTS: Clipping Weight

[1]Ca=Calcium K=Potassium

[2]E.C.=Electrical Conductivity

4. RESULTS AND DISCUSSION

4.1 WEIGHT vs TIME-EXPERIMENT 1

Riely *et al.* (1979) stated that, "measuring the annual growth of bryophytes is difficult because of their small size, delicate nature and the variety of growth patterns found in different species. It is also hard to decide what constitutes a single plant, and to determine the limits of a single year's growth." Riely *et al.* (1979) had difficulty monitoring moss growth, in particular, *Pleurozium*. The monitoring of moss growth, over time, was also a problem for Busby (1976) and Hettinger (1981; 1983) in their field experiments.

Various researchers have experienced difficulty in monitoring (Wilmot-Dear 1980; Furness and Grime 1982a) and growing (Wigglesworth 1947; Busby 1976; Longton and Greene 1979) mosses under controlled conditions in the greenhouse. Therefore Experiment 1, of the present study, was designed as a monitoring experiment to determine:

1. if moss could be grown successfully over a period of time in the greenhouse,
2. if the regrowth could be measured accurately, and
3. the approximate recovery period needed before measurements are taken.

This experiment could also be considered a 'control' experiment with eight regrowth sampling times instead of the one sampling time used in Experiments 2 to 6. Plants in

Experiment 1 were subjected to the same procedure (Table 3) as the other experiments except distilled water (0 mS/cm) was the only stress agent applied for seventy two hours.

4.1.1 REGROWTH BIOMASS

Forman (1969) compared coverage/m^2, energy/m^2, and biomass/m^2 as measures of bryophyte standing crop in various ecosystems. Coverage was regarded as a relatively poor measure of standing crop. Plasticity within bryophyte populations makes the use of shoot length as a measure of performance somewhat questionable. It was concluded energy/m^2, measured in Kcalories, was the best measure for general ecosystem purposes. However, in its absence, biomass/m^2 was a good estimate because curves for energy and biomass were very similar (Forman 1969).

Biomass/m^2 was used by Riely *et al.* (1979) in their study of the ecological role of bryophytes in an oak woodland in North Wales. Dry matter biomass was suggested as a measure of bryophyte performance, particularly if comparisons between populations in different environments are involved (Furness and Grime 1982a). In contrast to the three previous sources, Vitt and Pakarinen (1977) concluded that production cannot be estimated by use of phytomass (green moss portions), because both growth patterns and rates of decomposition vary between habitats.

In the present experiments moss regrowth was considered a reliable measure of experimental results (Mayo 1981,

personal communication). For reclamation purposes, biomass was regarded as the most important factor. Therefore regrowth biomass, in grams, was adopted as the term of expression for experimental results, rather than using a relative term. A relative term would express regrowth as a fraction or percentage of the total original moss biomass. In Experiment 1 a relative expression, regrowth percent (see Percent Regrowth), was used in addition to regrowth biomass. Percent regrowth was used to compare regrowth in the three feather mosses.

Standard error of the mean was used to measure variation throughout the experiments as response of the population was of interest, rather than response of individual plants. Tamm (1953) stated the standard deviations of each moss sample mean averaged about 20%, and the standard deviations of individual moss plants must still be higher. "Evidently the biological variation inherent in the *Hylocomium* community is much greater than the errors in the measurements" (Tamm 1953).

Monthly growth production is small compared with the existing total biomass (Busby 1976). In Experiment 1, moss tips were used instead of the longer moss segments to increase the proportion of regrowth biomass to original biomass. All bags contained the same number of tips (twenty five) and all were of equal length (20 mm) at the start of the experiment. *Hylocomium* tips were the heaviest, two to three times the weight of *Ptilium* or *Pleurozium*. *Ptilium*

tips had the lowest starting weight. Under the given conditions, *Hylocomium* gave the greatest amount of regrowth biomass and *Ptilium* the least (Figure 4) by both weighing methods. The statistical significance of these values are compared for Bag Weight Difference (Table 7) and for Clipping Weight (Table 8). 'Replicates' was the only variable that showed a difference in the analyses of the two weighing methods. In the Bag Weight Difference values, 'Replicates' was not significant whereas in the Clipping Weight values, 'Replicates' was highly significant ($P \leq 0.01$). Differences in regrowth values of 'Species', 'Time' and the 'Species X Time' interaction were highly significant ($P \leq 0.01$) in Experiment 1 (Tables 7 and 8).

Regrowth of *Hylocomium* appeared as a new 'segment' (Bengtson *et al.* 1982) or 'increment' (Tamm 1953; Busby 1976) throughout the experiments. In the field, annual growth of *Hylocomium* forms a new branch on the moss sympodium (Bengtson *et al.* 1982). Annual growth increments were used by Tamm (1953; 1964) to investigate seasonal growth, yield and nutrition of *Hylocomium*. Busby (1976) stated the cyclic changes in *Hylocomium* are not necessarily seasonal but reflect abrupt changes in temperature or water supply or both. Results of Experiment 1 would agree with this as there was a change of temperature, from the cooler chamber to the laboratory, and a change in water supply. In Experiments 2 to 6 contact with brine or sorbitol would also be considered an abrupt change for *Hylocomium*. Regrowth of

Hylocomium appeared as a new increment arising from the back of the previous season's segment.

It was speculated the decrease in regrowth biomass for *Ptilium* and *Pleurozium* at twenty two weeks (Figure 4) was related to the removal of shades from the greenhouse roof and the shut down of the cooling system in preparation for winter. There were a number of very warm sunny days after this, which caused a large increase in temperature and presumably a corresponding decrease in relative humidity. *Ptilium* regrowth and to a lesser extent *Pleurozium* regrowth tended to stick on the bag, when material was removed for clipping, which resulted in a lower value for the Clipping Weight than the Bag Weight Difference (Figure 4). The sticking problem was reduced somewhat, if material was removed while the bag was damp.

FIGURE 4: **REGROWTH** vs **RECOVERY TIME** of three feather mosses after **DISTILLED WATER STRESS** for three days. (EXPT. 1, GREEN TIPS, BAG CULTURE, TWO WEIGHING METHODS)

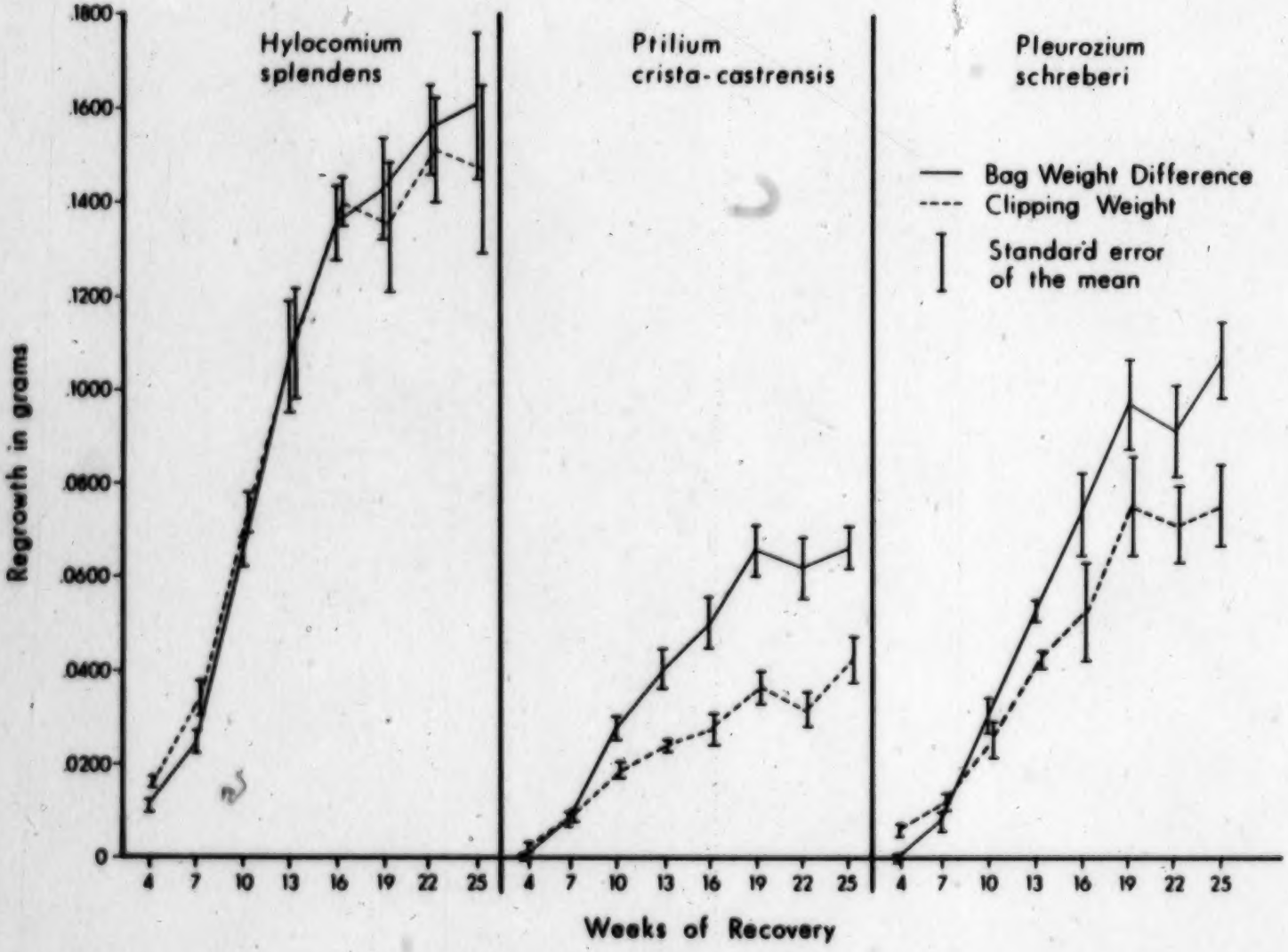

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Bag Weight Difference
Clipping Weight
Standard error of the mean
Regrowth in grams
.1800
.1600
.1400
.1200
.1000
.0800
.0600
.0400
.0200
0
4 7 10 13 16 19 22 25
Weeks of Recovery

TABLE 7: Analysis of variance, BAG WEIGHT DIFFERENCE Regrowth of three feather mosses after DISTILLED WATER stress for three days. Eight recovery times.

EXPT. 1, Green Tips, Bag Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.00318	0.00064	2.13	NS
Species (Spp.)	2	0.09072	0.04536	151.20	**
Time	7	0.22828	0.03261	108.70	**
Spp. X Time	14	0.02686	0.00192	6.40	**
Error	115	0.03398	0.00030	1.00	
Total	143	0.38302			

TABLE 8: Analysis of variance, CLIPPING WEIGHT Regrowth of three feather mosses after Distilled Water stress for three days. Eight recovery times.

EXPT. 1, Green Tips, Bag Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.00713	0.00143	4.93	**
Species (Spp.)	2	0.15273	0.07636	263.30	**
Time	7	0.12372	0.01767	60.93	**
Spp. X Time	14	0.03706	0.00265	9.14	**
Error	115	0.03369	0.00029	1.00	
Total	143	0.35433			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

4.1.2 PERCENT REGROWTH

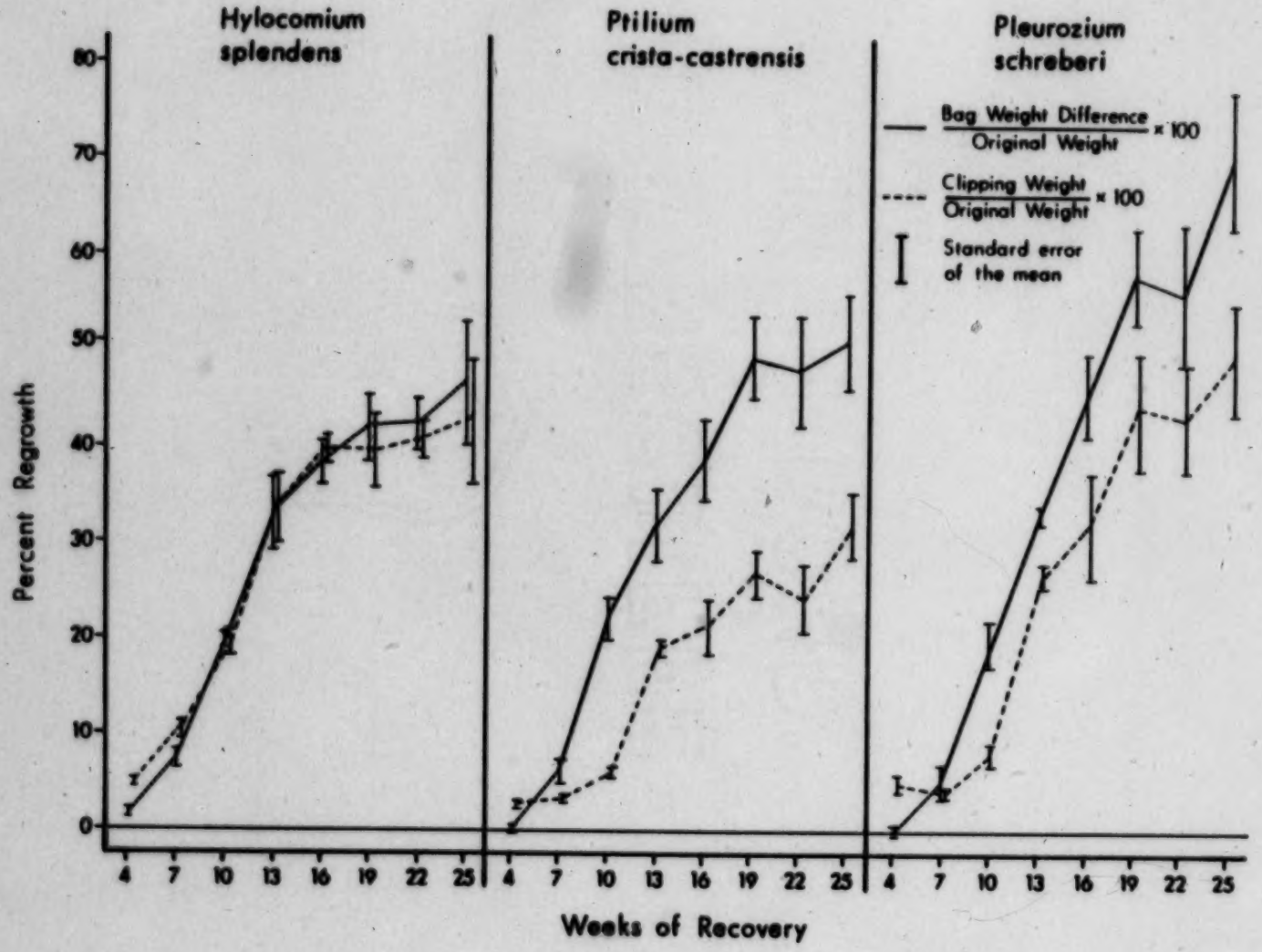
Furness and Grime (1982a; 1982b) compared the growth rates of forty species of bryophytes of contrasting growth forms and ecology, over a range of temperatures using the mean relative growth rate as an index. That is the slope of a fitted linear regression of $\log_{10}$ dry weight on time. To compare the three moss species of my Experiment 1, on a relative basis, regrowth was expressed as percent regrowth [(Bag Weight Difference/Original Moss Weight) X 100] or [(Clipping Weight/Original Moss Weight) X 100]. The gap between regrowth of *Hylocomium* and the other mosses in Figure 4 was closed considerably in Figure 5. Analysis of variance was not performed on the percent regrowth values but one can compare data by use of the standard errors. The relative growth of *Hylocomium* and *Pleurozium* was similar. Clipping Weight percentage values (dashed line) for *Hylocomium* and *Pleurozium* overlap except from seven to thirteen weeks. *Ptilium* Clipping Weight percentage values, the lowest of the three species, are different from those of *Hylocomium* and *Pleurozium* over the entire recovery period, except at nineteen weeks. At nineteen weeks the standard errors for *Ptilium* and *Pleurozium* overlap.

Experiment 1 proved moss could be grown successfully over a period of time in the greenhouse. *Hylocomium* seemed well suited to Bag Culture in this experiment. The two weighing methods (Bag Weight Difference and Clipping Weight) produced almost the same values for *Hylocomium*, also the

trends were the same for *Pleurozium* and to a lesser degree for *Ptilium*. The difference between the two methods for *Ptilium* and *Pleurozium* was the result of moss regrowth sticking to the bags, as explained previously. It was necessary to wait for seven weeks of recovery before a positive value was recorded with the Bag Weight Difference. The curves start to level off after sixteen weeks. Experiment 1 was under 'ideal' conditions therefore it was decided at least sixteen weeks of recovery would be needed before measuring regrowth in the next experiments because mosses would be subjected to conditions that were far from ideal. Furness and Grime (1982b) stated that long-term growth experiments appear to present fewer problems.

A strong odor was detected, after the distilled water flush in Experiment 1, which led to a fear that regrowth may not occur. The odor suggested, there was a considerable amount of microbial activity during the time of stress application. Also the control treatments, distilled water (0 mS/cm equivalent) were the most odoriferous throughout Experiments 2 to 5. Experiment 6 did not include a distilled water control. Microbes were artifacts in Gupta's (1977) studies of respiration responses of bryophytes to desiccation.

FIGURE 5: **PERCENT REGROWTH** vs **RECOVERY TIME** of three feather mosses after **DISTILLED WATER STRESS** for three days. (EXPT. 1, GREEN TIPS, BAG CULTURE, TWO WEIGHING METHODS)


Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Bag Weight Difference / Original Weight x 100
Clipping Weight / Original Weight x 100
Standard error of the mean
Percent Regrowth
0
10
20
30
40
50
60
70
80
4 7 10 13 16 19 22 25
Weeks of Recovery

4.2 COMPARISON OF TECHNIQUES

Experimental techniques compared in Experiments 1 to 6 were as follows:

1. two types of culture, Bag and Net
2. two weighing methods, Bag Weight Difference and Clipping Weight
3. two moss storage methods, wet and dry
4. two moss lengths, tips and segments
5. two colors of moss samples, brown and green
6. three sources of moss samples; Swan Hills 1980, Swan Hills 1981, and Villeneuve 1981.

The topics of culture methods, weighing methods, and brown versus green moss are expanded further in later sections. See the Summary of Experiments at the end of Methods and Materials for more details. The sequence of procedures was outlined in Table 3.

Two methods of culture were compared in the first four experiments. Experiments 1 and 3 used Bag Culture with polyester bags. The stress (Table 3) agent used in Experiment 1 was distilled water only. Two stress agents, brine or sorbitol at five concentration levels, were used in Experiment 3. The effect of varied stress agents on the weighing methods is presented later in the Comparison of Weighing Methods section. Regrowth weight was measured by two different methods Bag Weight Difference and Clipping Weight in Experiments 1 and 3. In the Bag Weight Difference both polyester bag and moss contents were weighed at the

beginning and at the end of the recovery period. To determine the Clipping Weight, regrowth was removed from the original moss after the recovery period. Clippings were put into small pellon bags (Plate 5) and stored at a relative humidity of 20% before weighing. This relative humidity was obtained by using a saturated potassium acetate solution in the holding cabinets. Unlike the larger polyester bags, pellon bags were used for weighing regrowth only. Pellon bags were NOT used in the greenhouse during the recovery period. Only green moss tips were used in Experiments 1 and 3, however the source and storage method of samples varied. Experiment 1 used dry storage samples collected from Villeneuve 1981, whereas Experiment 3 used wet storage samples collected from Swan Hills in 1980.

Experiments 2 and 4 used Net Culture (Plate 6) with brine or sorbitol applied as the stress agents. Unlike Experiments 1 and 3, mosses were placed directly in plastic inserts in a horizontal position for recovery, and only the Clipping Weight was used to measure regrowth. Net Culture was used to compare brown and green moss segments. Brown moss segments (Experiment 2) were from the wet storage samples, collected from Swan Hills 1980 (same source as Experiment 3 samples). Green segments (Experiment 4) were dry storage samples collected from Villeneuve 1981 (same source as Experiment 1 samples).

4.2.1 BAG CULTURE

Bag Culture was a very time consuming method in terms of preparation and procedure. In an attempt to reduce extraneous weight losses or gains, both bags and moss samples required extra preparation time. Polyester bag material had to withstand the recovery period, which likely included a certain amount of microbial action. By the end of Experiment 3, thread was starting to disintegrate on some of the bags. Algae was a greater problem in Experiment 3 than in Experiment 1. Bags remained in the vials for the recovery period in both experiments and there was a tendency for water to accumulate in the vials. Placing the bag directly in the plastic cell would have alleviated the water accumulation as there were drainage holes in both the plastic insert cells and the large holding trays. This may have reduced the growth of algae but the loss of moss fragments may have increased with free movement of water through the bags during misting operations. These potential losses and gains in weight would be a concern if the Bag Weight Difference was used for measurement of regrowth. Bags were weighed after the water flush because moss fragments were lost during the stress and flush treatments. The negative values for *Ptilium* and *Pleurozium* at four weeks in Figure 5 indicate more fragments were lost when the bags were misted during the recovery period.

Hylocomium seems well suited to Bag Culture if the 'starching effect' is avoided by not using concentrated

solutions such as sorbitol and brine, and the recovery period is short enough to prevent problems from algae (expanded further in the next section). Regrowth can be expressed in terms of biomass increase as well as percentage increase, compared to the original weight. Invaders were kept to a minimum with Bag Culture compared to Net Culture.

4.2.2 COMPARISON OF WEIGHING METHODS

Bag Weight Difference and Clipping Weight were the two weighing methods compared in Experiments 1 and 3. They were used to measure the same plant samples, which led me to believe a paired Student T-test could be used to test for a significant difference between the two methods. Statistical data for Experiments 1 and 3 are presented in Table 9 including T values and their significance levels, and r^2 values for Bag Weight Difference and Clipping Weight. The two methods are also depicted in Figure 4 for Experiment 1 and Figures 6 and 7 for Experiment 3.

The only place where the T values, r^2 values and figures agreed was for *Hylocomium* in Experiment 1. Variations between the species, and large positive and negative values tend to make the T-test questionable. In my opinion the r^2 values and figures were more reliable than the T-test. Steel and Torrie 1960 suggested that pairs need to be positively correlated for the T-test to be effective. The r^2 values and T values (Table 9) indicate this was true.

The two weighing methods gave similar values (Figure 4) and were highly correlated (r^2 values, Table 9) in Experiment 1 because only distilled water was used as the stress agent. Bag Weight Difference was an unsatisfactory method of measurement in Experiment 3 because brine and sorbitol had the same effect as laundry starch on the bags, sorbitol more so than brine. In my preliminary experiments, brine deposits on moss tips were easier to remove with a water rinse than sorbitol deposits. The concentrated solutions adhered to the bags to make them stiff and also added extra weight to the bags during the stress treatments. Likely more brine than sorbitol was removed from the bags during the distilled water flush. Bags in sorbitol treatments probably lost more weight from the misting operations during the recovery period, and therefore lead to the poor correlation of the Bag Weight Difference and Clipping Weight. The largest negative Bag Weight Difference values occurred in the sorbitol treatments with the highest concentration levels (40 mS/cm equivalent) for all three species (Table 9, Figure 7).

TABLE 9: Comparison of Bag Weight Difference and Clipping Weight of three feather mosses after a three day stress: Distilled Water (EXPT. 1), Brine or Sorbitol (EXPT. 3)

PAIRS	SPECIES[1]	STRESS[2]	EXPT.	r^2	T-TEST	
144	All	DW	1	.89	6.47	**
180	All	B + S	3	.10	0.97	NS
48	Hs	DW	1	.95	-0.57	NS
48	Pcc	DW	1	.72	6.96	**
48	Ps	DW	1	.89	5.30	**
90	All	B	3	.18	3.29	**
90	All	S	3	.08	-1.23	NS
60	Hs	B + S	3	.06	-2.48	*
60	Pcc	B + S	3	.24	3.23	**
60	Ps	B + S	3	.17	2.83	**

[1]SPECIES: Hs= *Hylocomium splendens*
Pcc=*Ptilium crista-castrensis*
Ps= *Pleurozium schreberi*

[2]STRESS: DW=Distilled Water, B=Brine, S=Sorbitol

NS=Not Significant
*=Significant ($P \leq 0.05$) **=Highly Significant ($P \leq 0.01$)

FIGURE 6: **COMPARISON OF WEIGHING METHODS.**
Regrowth vs Stress Level of three feather mosses after **BRINE STRESS** for three days.
BAG WEIGHT DIFFERENCE vs **CLIPPING WEIGHT** after twenty two weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 3, GREEN TIPS, BAG CULTURE)

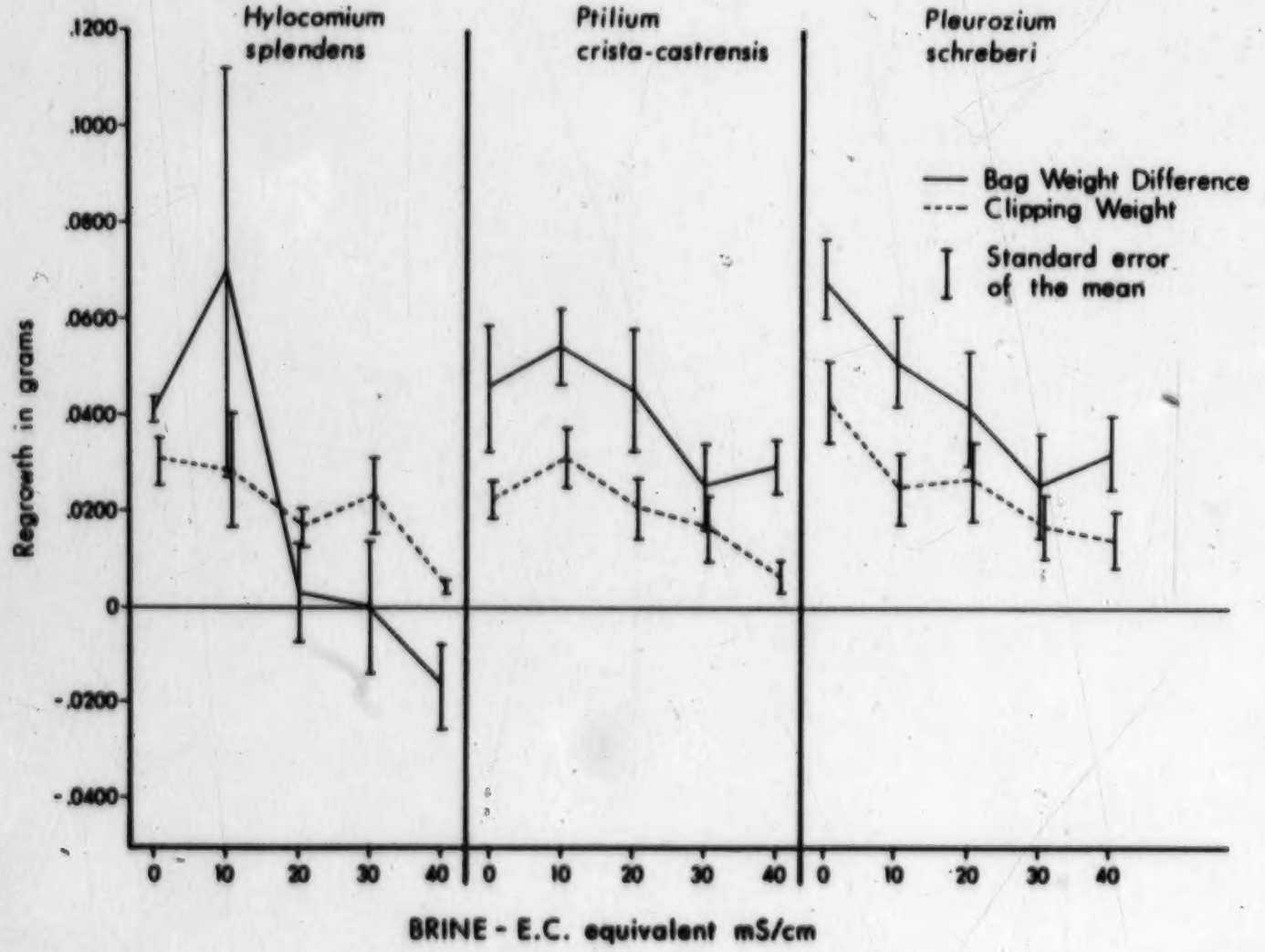
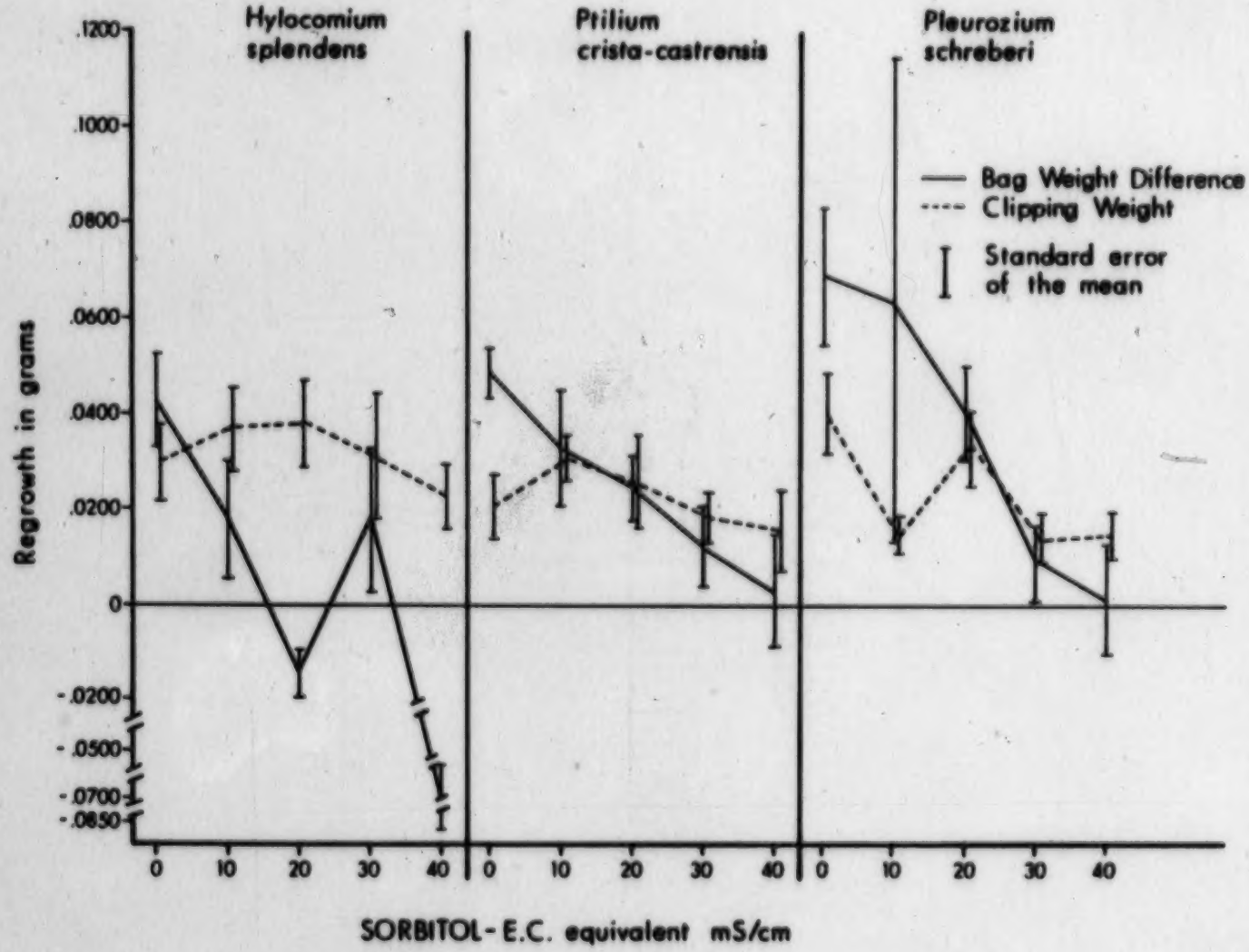

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Bag Weight Difference
Clipping Weight
Standard error of the mean
Regrowth in grams
.1200
.1000
.0800
.0600
.0400
.0200
0
-.0200
-.0400
0
10
20
30
40
BRINE - E.C. equivalent mS/cm

FIGURE 7:

COMPARISON OF WEIGHING METHODS.
Regrowth vs Stress Level of three feather mosses after **SORBITOL STRESS** for three days.
BAG WEIGHT DIFFERENCE vs **CLIPPING WEIGHT** after twenty two weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 3, GREEN TIPS, BAG CULTURE)


Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Bag Weight Difference
Clipping Weight
Standard error of the mean
Regrowth in grams
.1200
.1000
.0800
.0600
.0400
.0200
0
-.0200
-.0500
-.0700
-.0850
0
10
20
30
40
SORBITOL - E.C. equivalent mS/cm

Three artifacts contributed to errors with Bag Culture.

1. Regrowth stuck on the bags thus giving lower Clipping Weight values for *Ptilium* and to a lesser extent for *Pleurozium*.
2. Bag Weight Difference values were affected by loss of moss particles, which occurred any time the bags were in contact with a liquid e.g. stress application, misting.
3. Growth of algae on the bags probably increased Bag Weight Difference. Removal of algae by rinsing likely created a loss of moss particles which decreased Bag Weight Difference.

Clipping Weight was the preferred method of measuring regrowth, especially in Experiment 3. Standard errors of the mean do not vary to the same degree as those in the Bag Weight Difference, and there are no negative means. A magnifying glass and adequate lighting were necessary to prevent eye strain during the clipping process. Regrowth tended to break at the junction of old and new growth, aiding in separation of the two fractions. Also regrowth usually appeared as a lighter, more translucent shade of green. Removal of regrowth was very tedious, but the Clipping Weight method avoided the extraneous losses and gains experienced with the Bag Weight Difference.

4.2.3 NET CULTURE

Net Culture (Plate 6) reduced preparation time greatly. Disks were cut out of the porous net material, and no sewing

was needed. Only one weighing session was required instead of the several weighing sessions required for Bag Culture (See Table 3). Moss segments were used instead of tips, and detailed cleaning and trimming were not required as only regrowth was weighed. The option of expressing regrowth as a percentage of the original moss weight was lost with Net Culture. If invader species are present in large numbers, regrowth biomass would be the desireable term of expression. Expressing regrowth as a percentage of the original moss weight, should be used with caution since weights include both original species and invaders.

The 'starching effect', or algae posed no problems with the porous net material. Although algae may have been responsible for the occassional loss of a moss segment, leaves fell off and only the wiry skeleton remained. Unlike Bag Culture, moss bundles were placed directly in the plastic cells in a horizontal position. The presence of drainage holes in the cells and trays eliminated the water accumulation problem experienced with the vials. Original plants were in a horizontal position therefore, regrowth was at 90°, which aided in the clipping process. Regrowth in Net Culture experiments had a close resemblance to the appearance of plants found in the field. By the end of the recovery period several cells looked like moss miniplots.

4.2.4 BROWN vs GREEN MOSS

Green moss tips of 20 mm were used in Experiments 1 and 3 to reduce the amount of original material to a minimum and thereby make detection of regrowth with the Bag Weight Difference easier. Green segments were used in Experiment 4 where Clipping Weight was used to measure regrowth. If any samples had twin tips, one tip was removed before the start of the experiment. In these experiments 'segment' refers to a length of moss with a maximum length of 5 cm at the start of the experiment. 'Segment', in this case, does not have the same connotation as that used by Bengston *et al.* (1982) in their study of *Hylocomium splendens*. The authors defined a segment length as "the distance from the point where it emerged from the mother segment to the point on its stem where the following year's segment was attached."

Brown segments were used in Experiment 2 because the green regrowth could be readily distinquished from the original brown moss, thus aiding the separation of old and new growth. Brown segments were more brown colored than green, actually the rejected material from Experiment 3. The brown discoloration occurred because samples were put directly in the cooler (Swan Hills 1980) instead of being air dried first then put in the cooler (Swan Hills 1981, Villeneuve 1981). Samples with brown discoloration had been in the cooler for more than ten months compared to the one month old samples used in Experiment 1. In reference to moss samples, Lee and Stewart (1971) mentioned the "deleterious

effects of prolonged storage in a saturated atmosphere." *Hylocomium* discolored much faster and to a greater extent than *Ptilium* or *Pleurozium*. *Hylocomium* was missing from Experiment 2 because the brown material had already been discarded. *Ptilium* showed the least amount of brown discoloration of the wet storage samples.

Since brown moss is usually considered dead or inactive (Vitt and Pakarinen 1977; Bates 1979), three questions arose about the brown segments.

1. Would there be any regrowth on the brown segments?
2. If so, would there be a difference in regrowth because of reduced vitality?
3. Would the response of the brown segments, to the stress agents (brine or sorbitol), be the same as that of the green segments or tips?

It was concluded that a brown moss was not necessarily an inactive moss as regrowth did occur (Figure 8), however the amount of regrowth was low compared to the amount for green tips (different source) at sixteen weeks in Figure 4. Green tips in Figure 9 were from the same source as the brown segments. The general response trends for brown segments (Figure 8) and green segments (Figure 10) to brine and sorbitol were somewhat similar, more so than those in Figure 9. I believe the different response trend in Figure 9 was caused by procedure rather than the particular moss sample. This idea is expanded further in the Brine/Sorbitol section (Salt Tolerance vs Drought Tolerance).

FIGURE 8: **REGROWTH vs STRESS LEVEL** of two feather mosses after **BRINE** or **SORBITOL STRESS** for three days.
Clipping Weight after seventeen weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 3, BROWN SEGMENTS, NET CULTURE)

FIGURE 9: **REGROWTH vs STRESS LEVEL** of two feather mosses after **BRINE** or **SORBITOL STRESS** for three days.
Clipping Weight after twenty two weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 3, GREEN TIPS, BAG CULTURE)

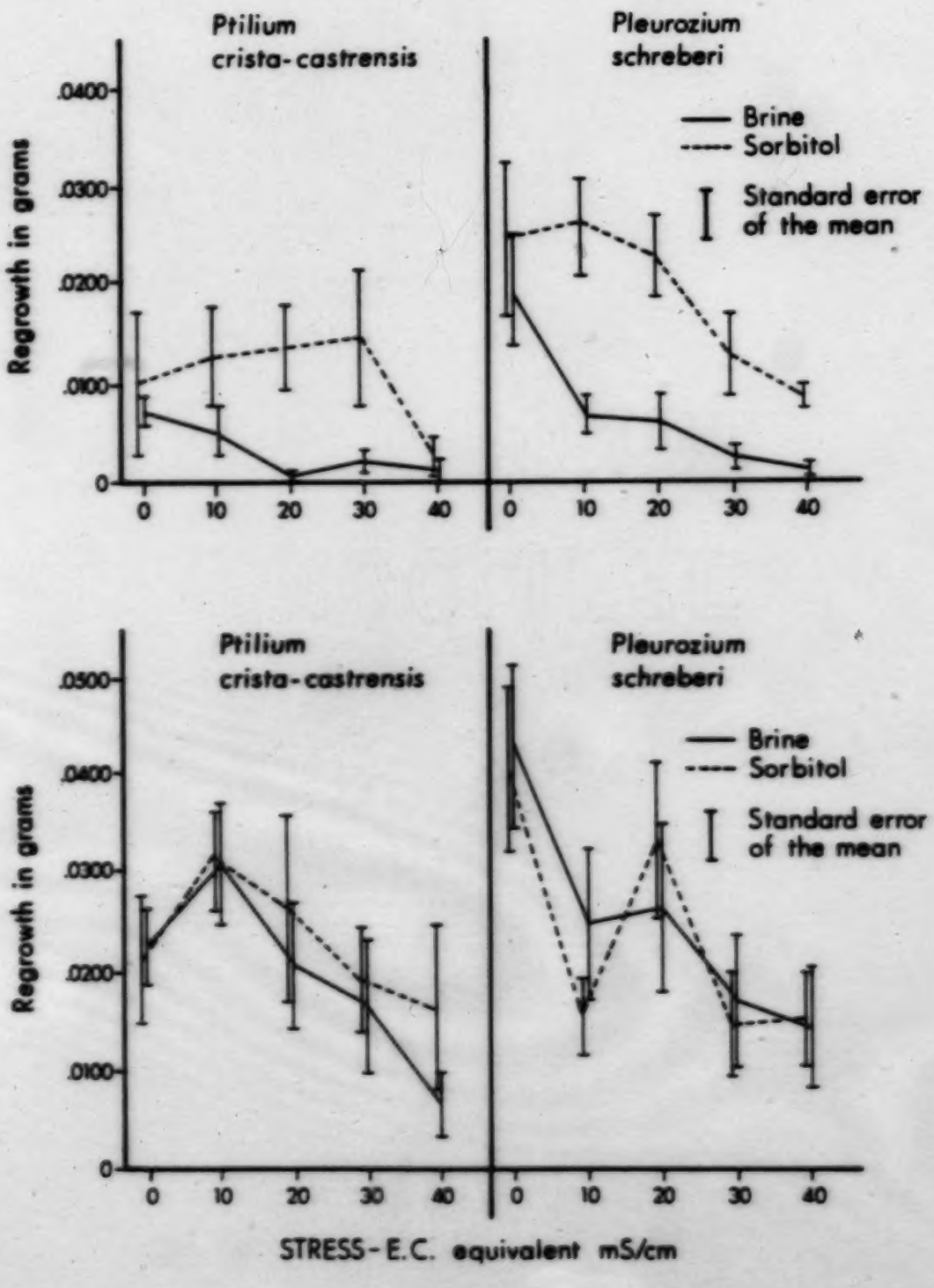

Ptilium
crista-castrensis
Pleurozium
schreberi
Brine
Sorbitol
Standard error
of the mean
Regrowth in grams
.0400
.0300
.0200
.0100
0
0
10
20
30
40
Ptilium
crista-castrensis
Pleurozium
schreberi
Brine
Sorbitol
Standard error
of the mean
Regrowth in grams
.0500
.0400
.0300
.0200
.0100
0
0
10
20
30
40
STRESS-E.C. equivalent mS/cm

FIGURE 10: **REGROWTH** vs **STRESS LEVEL** of three feather mosses after **BRINE** or **SORBITOL STRESS** for three days. **CLIPPING WEIGHT** after thirty weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 4, GREEN SEGMENTS, NET CULTURE)

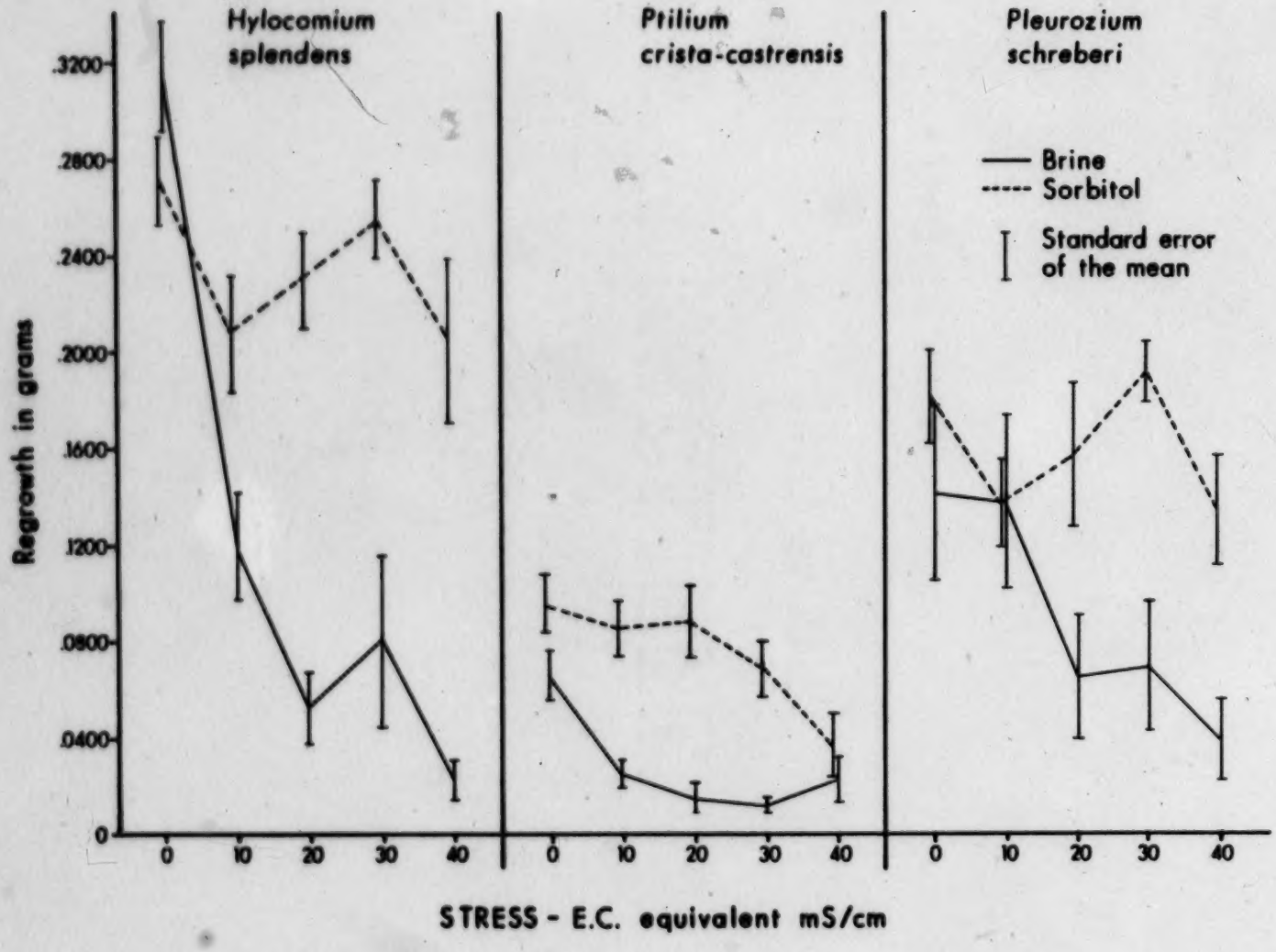

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Brine
Sorbitol
Standard error of the mean
Regrowth in grams
.3200
.2800
.2400
.2000
.1600
.1200
.0800
.0400
0
0
10
20
30
40
STRESS - E.C. equivalent mS/cm

FIGURE 11: **REGROWTH vs STRESS LEVEL** of three feather mosses after **BRINE or SORBITOL STRESS** for three days. **BAG WEIGHT DIFFERENCE** after twenty two weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 3, GREEN TIPS, BAG CULTURE)

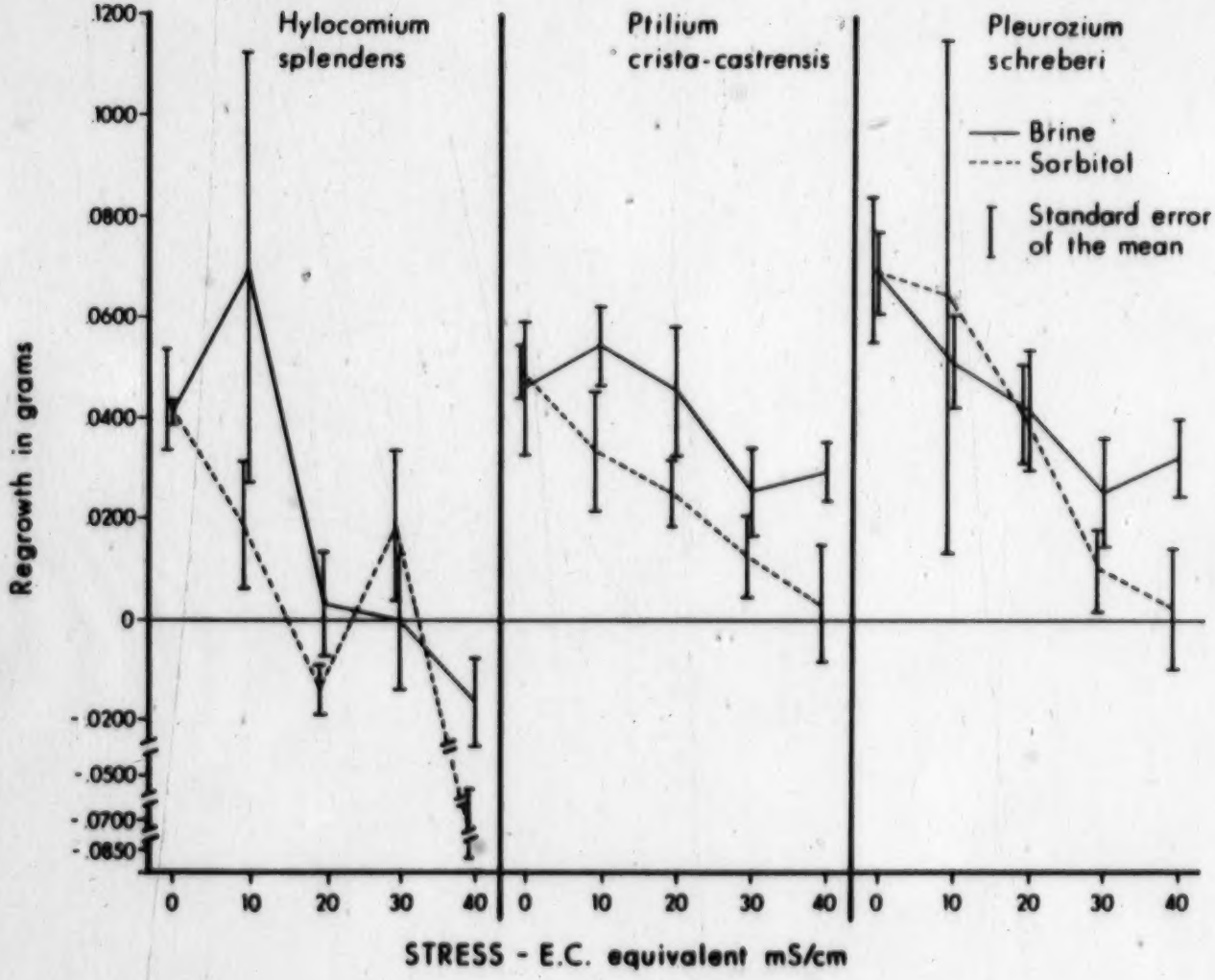
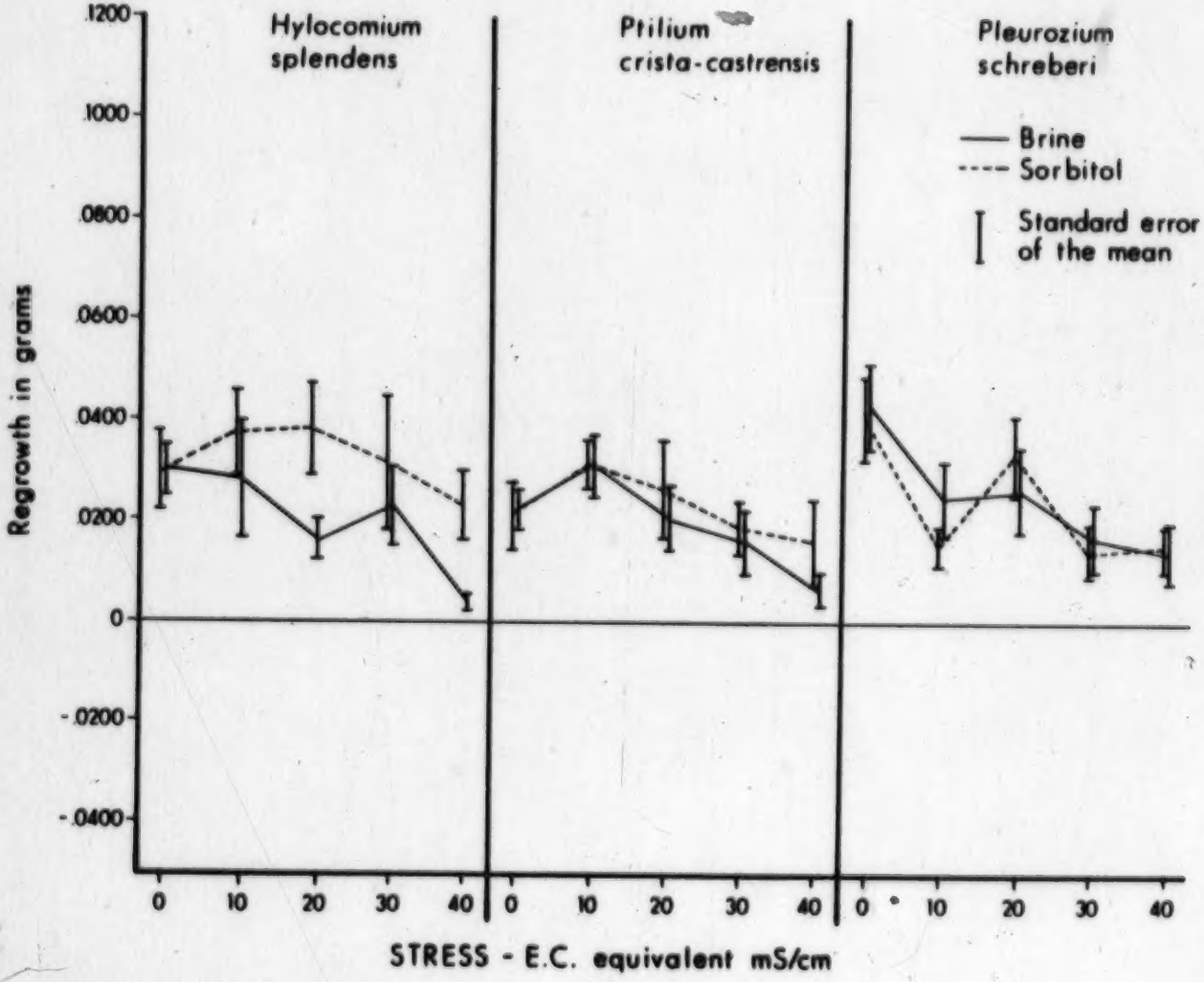

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Brine
Sorbitol
Standard error of the mean
Regrowth in grams
.1200
.1000
.0800
.0600
.0400
.0200
0
-.0200
-.0500
-.0700
-.0850
0
10
20
30
40
STRESS - E.C. equivalent mS/cm

FIGURE 12: **REGROWTH vs STRESS LEVEL** of three feather mosses after **BRINE or SORBITOL STRESS** for three days. **CLIPPING WEIGHT** after twenty two weeks recovery.
Control=0 mS/cm equivalent E.C.=Electrical Conductivity
(EXPT. 3, GREEN TIPS, BAG CULTURE)


Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Brine
Sorbitol
Standard error of the mean
Regrowth in grams
.1200
.1000
.0800
.0600
.0400
.0200
0
-.0200
-.0400
0
10
20
30
40
STRESS - E.C. equivalent mS/cm

4.2.5 INVADERS

Regrowth biomass, in both Experiments 2 and 4 (Figures 8 and 10 respectively), included regrowth of the original species plus growth from moss invaders. Separation of invaders from original moss was considered but it was too time consuming because invaders were interspersed with the original species. Sorting invaders and original species would involve identifying each shoot which would be very time consuming and tedious. Loss of regrowth fragments from excessive handling was also a concern.

The presence of invaders in large numbers, was first noted in Experiment 2. Invaders were mainly *Pohlia nutans* with some *Sphagnum* species, *Aulacomnium palustre*, *Funaria hygrometrica* and *Bryum* species. In Experiment 4, *Aulacomnium palustre* was the main invader, followed by *Bryum* species, with some *Campylium* species, *Ceratodon purpureus*, *Pohlia nutans* and *Funaria hygrometrica*. Most of the colonizing species involved were so called 'weedy species' (Vitt 1982, personal communication). *Aulacomnium palustre* was one of the species that invaded Studlar's (1983) field plots which monitored moss recovery from trampling.

Invaders were more prevalent in the brown segments than in the green segments. There was no noticeable correlation between stress levels and invaders, or stress agent and invaders. However there was a correlation, in both experiments, between original moss species and invaders. The largest occurrence of invaders were found on *Ptilium*,

followed by *Pleurozium*. The closely spaced leaves of *Ptilium* (Plates 1 and 3) provided a moist, flat surface for colonization by spores that happened to land. The presence of invaders, in these experiments, was a reason for concern as individual species were being compared. However the possible use of colonization as a reclamation measure, may be the most significant idea, in terms of field application. Moss samples from unaffected areas near a spill site could be pulverized and added to the final flush water. This method of 'seeding' an affected area could be easily incorporated into the present reclamation procedure described by Innes and Webster 1980b. However, this idea has not been tested in the field, as yet (Ross and Webster 1983). If seeding was successful, it is possible certain species could be selected and stored for reclamation use. Bayfield (1976) compared growth of moss fragments (samples were pulverized) on several types of leaf litter and growing media in a greenhouse experiment.

4.3 SALT TOLERANCE vs DROUGHT TOLERANCE-EXPERIMENTS 2 to 4

There was an indication, from my preliminary experiments, that moss response to mannitol and brine varied, however they had not been tested together at the same time. Sorbitol was selected for these experiments because it was cheaper than mannitol, and reported to be very soluble, nontoxic and an efficient osmotic solute. It is reportedly slightly less inhibitory of enyzme activity

than mannitol and forms a solution with pH 7 (Brown and Hellebust 1978). Sorbitol occurs naturally in some plants to counteract the effects of saline environments (Ahmad *et al.* 1979).

Experiments 2, 3 and 4 (Figures 8, 11 and 12, and 10) compared moss response, measured by regrowth biomass, to brine and sorbitol. All three experiments had the same stress levels (electrical conductivity or its equivalent, in the case of sorbitol) and contact time. Moss length (tips vs segments) and culture (Bag vs Net) varied in these three experiments. The difference between the two stress agents was highly significant ($P \leq 0.01$) in Experiments 2 and 4 (Tables 10 and 13) but not significant ($P \leq 0.05$) in Experiment 3 (Tables 11 and 12). Interactions were not significant in Experiments 2 and 3. However in Experiment 4 (Table 13), the interactions were highly significant, including the triple interaction of 'Species X Agents X Level'. This meant each moss reacted differently to brine and sorbitol, and response changed as the concentration level of stress changed (Figure 10). The response patterns to brine and sorbitol were similar for *Hylocomium* and *Pleurozium* in this experiment. The difference between the stress agents was closer at the lower mS/cm equivalents, becoming wider, then stayed about the same distance from one another at the higher mS/cm equivalents. In *Ptilium*, the gap between sorbitol and brine first increased up to 20 mS/cm equivalent, then narrowed to the point of no significant

difference at 40 mS/cm equivalent (standard errors overlapped). Sorbitol, the osmotic effect similar to drought (Greenway 1973), had no significant effect (standard errors overlapped) on the feather mosses up to 30 mS/cm equivalent (Figure 10). *Ptilium* regrowth exhibited the largest decrease of the three mosses from 0 to 40 mS/cm equivalent of sorbitol. Unlike *Hylocomium* and *Pleurozium* (Figures 10 and 12), the drought and salt tolerance of *Ptilium* were the same at 40 mS/cm equivalent. *Ptilium* and *Hylocomium* reacted to brine, in a similar manner in Experiment 4 (Table 16, Figure 10) with a large decrease between 0 and 10 mS/cm. But in contrast, *Hylocomium* experienced a further marked drop in regrowth between 30 and 40 mS/cm of brine while *Ptilium* regrowth increased slightly between these two levels. *Pleurozium* regrowth decreased markedly between 10 and 20 mS/cm in the brine treatments. *Pleurozium* showed its variable character with a different response pattern in all three experiments. In field studies of brine spills, Hettinger (1980) found the response of *Pleurozium* to be the most variable of the three feather mosses.

TABLE 10: Analysis of variance, CLIPPING WEIGHT
Regrowth of two feather mosses after Brine/Sorbitol for three days. Seventeen weeks recovery.

EXPT. 2, Brown Segments, Net Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.00087	0.00017	1.70	NS
Species (Spp.)	1	0.00124	0.00124	12.40	**
Stress Agents	1	0.00275	0.00275	27.50	**
Stress Levels	4	0.00213	0.00053	5.30	**
Spp. X Agents	1	0.00013	0.00013	1.30	NS
Spp. X Level	4	0.00071	0.00018	1.80	NS
Agents X Level	4	0.00063	0.00016	1.60	NS
Spp. X Agents X Level	4	0.00012	0.00003	0.30	NS
Error	95	0.00953	0.00010	1.00	NS
Total	119	0.01811			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

TABLE 11: Analysis of variance, BAG WEIGHT DIFFERENCE Regrowth of three feather mosses after Brine/Sorbitol for three days. Twenty two weeks recovery.

EXPT. 3, Green Tips, Bag Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.00324	0.00065	0.42	NS
Species (Spp.)	2	0.03145	0.01572	10.08	**
Stress Agents	1	0.00957	0.00957	6.13	*
Stress Levels	4	0.08286	0.02071	13.28	**
Spp. X Agents	2	0.00153	0.00077	0.49	NS
Spp. X Level	8	0.01868	0.00233	1.49	NS
Agents X Level	4	0.00961	0.00240	1.54	NS
Spp. X Agents X Level	8	0.00831	0.00104	0.67	NS
Error	145	0.22552	0.00156	1.00	NS
Total	179	0.39075			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

TABLE 12: Analysis of variance, CLIPPING WEIGHT
Regrowth of three feather mosses after Brine/Sorbitol for three days. Twenty two weeks recovery.

EXPT. 3, Green Tips, Bag Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.00443	0.00089	2.87	*
Species (Spp.)	2	0.00084	0.00042	1.35	NS
Stress Agents	1	0.00097	0.00097	3.13	NS
Stress Levels	4	0.00751	0.00188	6.06	**
Spp. X Agents	2	0.00130	0.00065	2.10	NS
Spp. X Level	8	0.00398	0.00050	1.61	NS
Agents X Level	4	0.00124	0.00031	1.00	NS
Spp. X Agents X Level	8	0.00039	0.00005	0.16	NS
Error	145	0.04466	0.00031	1.00	NS
Total	179	0.06532			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

TABLE 13: Analysis of variance, CLIPPING WEIGHT
Regrowth of three feather mosses after Brine/Sorbitol for three days. Thirty weeks recovery.

EXPT. 4, Green Segments, Net Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.03566	0.00713	2.79	*
Species (Spp.)	2	0.47200	0.23600	92.19	**
Stress Agents	1	0.27204	0.27204	106.27	**
Stress Levels	4	0.20454	0.05113	19.97	**
Spp. X Agents	2	0.03720	0.01860	7.27	**
Spp. X Level	8	0.08045	0.01006	3.93	**
Agents X Level	4	0.07989	0.01997	7.80	**
Spp. X Agents X Level	8	0.06929	0.00866	3.38	**
Error	145	0.37168	0.00256	1.00	
Total	179	1.62275			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

On a percentage basis (Tables 15 and 16), *Pleurozium* and *Hylocomium* regrowth levels were very similar in the sorbitol treatments, suggesting they may have similar drought tolerances. Achuff (1974), Achuff and La Roi (1977), and Lee and La Roi (1979) had *Ptilium* and *Pleurozium* together in one group and *Hylocomium* in another group in their studies of forest communities. According to Achuff (1974) and Achuff and La Roi (1977) the *Rubus pedatus/Ptilium* community, which included *Pleurozium*, was associated with higher precipitation, cooler temperatures, and a lower soil nutrient status than the *Viburnum/Hylocomium* community type. The *Viburnum/Hylocomium* community was drier and warmer with richer soils. In Banff, Lee and LaRoi (1979) found that *Hylocomium* was found in a wider range of habitats, in terms of moisture, than *Ptilium* or *Pleurozium*. Their diagrams of *Ptilium* and *Pleurozium* moisture habitats are very similar.

TABLE 14: Percent Regrowth (based on control) of two feather mosses after Brine/Sorbitol for three days. Clipping Weight, seventeen week recovery.

EXPT. 2, Brown Segments, Net Culture

SPECIES[1]	STRESS[2]	0	10	20	30	40
Pcc	B	100	73	10	29	16
Pcc	S	100	128	138	146	27
Ps	B	100	47	31	13	6
Ps	S	100	107	98	52	35

TABLE 15: Percent Regrowth (based on control) of three feather mosses after Brine/Sorbitol for three days. Clipping Weight, twenty two week recovery.

EXPT. 3, Green Tips, Bag Culture)

SPECIES[1]	STRESS[2]	0	10	20	30	40
Hs	B	100	94	54	77	14
Hs	S	100	124	127	105	77
Pcc	B	100	137	92	73	29
Pcc	S	100	147	124	90	77
Ps	B	100	58	61	39	33
Ps	S	100	38	82	36	38

[1]SPECIES Hs= *Hylocomium splendens*
Pcc=*Ptilium crista-castrensis*
Ps= *Pleurozium schreberi*
[2]STRESS B=BRINE, S=SORBITOL in mS/cm equivalent

TABLE 16: Percent Regrowth (based on control) of three feather mosses after Brine/Sorbitol for three days. Clipping Weight, thirty week recovery.

EXPT. 4, Green Segments, Net Culture

SPECIES[1]	STRESS[2]	0	10	20	30	40
Hs	B	100	38	17	26	7
Hs	S	100	76	85	94	76
Pcc	B	100	37	22	17	34
Pcc	S	100	90	92	72	38
Ps	B	100	98	46	49	28
Ps	S	100	76	87	106	74

[1]SPECIES Hs= *Hylocomium splendens*
Pcc=*Ptilium crista-castrensis*
Ps= *Pleurozium schreberi*
[2]STRESS B=BRINE, S=SORBITOL in mS/cm equivalent

Based on all three experiments, it was concluded that brine had a greater detrimental effect on moss regrowth than sorbitol. The omotic plus specific ion effects were more harmful than the osmotic effect alone. The three mosses are less salt tolerant than drought tolerant. *Ptilium* was the least drought tolerant species and *Hylocomium* was the most salt sensitive of the three feather mosses. *Hylocomium* was found to be the most salt sensitive species by Hettinger (1980).

4.4 BRINE-EXPOSURE TIME vs LEVEL-EXPERIMENT 5

A seventy two hour exposure time was used for the five other experiments, as previous greenhouse experiments were based on this exposure time. This contact period was selected because a three or four day lag period would likely occur in the field from the time the spill occurs until a reclamation procedure can be initiated (Webster 1980). However, in Experiment 5 the time factor (two to ten days) versus the brine level (0 to 60 mS/cm) was investigated to ascertain if one was more important than the other. It was also of interest to determine if there was a particular limiting point, beyond which, further damage was minimal. Results were presented as Regrowth vs Exposure Time (Figure 13) and Regrowth vs Brine Level (Figure 14).

The main effects 'Species', 'Levels', and 'Time'(Table 17) are highly significant ($P \leq 0.01$). The 'Species X Level' interaction was also highly significant while the 'Species X Time' interaction was not significant. This indicated the brine concentration was more important than the contact time. If this relationship remains true under field conditions, reduction of the brine concentration by water flushing would aid moss recovery. This finding (flushing reduced damage) agreed with the findings of the field experiments of Edwards and Blauel (1975), the greenhouse experiments of Ross Daley (1980), and the field experiments of Innes and Webster (1980a), Webster *et al.* (1983).

In *Hylocomium* and *Ptilium* there was a significant difference between regrowth at 20 and 40 mS/cm but no significant difference (standard errors overlapped) between 40 and 60 mS/cm (Figure 13). These boundaries agreed with the findings of the Brine/Sorbitol Experiments 2 to 4 (Figures 8, 12 and 10), where the sharp drop in regrowth occurred between 30 and 40 mS/cm.

The greatest reduction of moss regrowth occurred between two and six days of exposure, in most cases (Figure 13). After six days of exposure to levels above 0 mS/cm, the amount of recovery was similar, especially for the 40 and 60 mS/cm levels. The reason for the sharp drop in regrowth of *Ptilium* controls (0 mS/cm) between eight and ten days (Figure 13) was not clear. It is possible ion leakage (Brown and Buck 1978) or microorganisms were a factor. Microbes may account for the lower value in *Hylocomium* at eight days also. The large drop in regrowth of *Hylocomium* between 0 and 20 mS/cm (Figures 13 and 14) indicate it was the most salt sensitive of the species. *Ptilium* was the most salt tolerant species, especially at the higher brine levels. In this experiment *Ptilium* was the most variable as values increased and decreased over time in a rather erratic manner, whereas the lines for *Hylocomium* (Figure 13) do not cross one another. The limiting point, where changes are minimal thereafter, appeared to be a six day exposure time and a brine level between 20 and 40 mS/cm.

FIGURE 13: **REGROWTH** vs **EXPOSURE TIME** of three feather mosses after BRINE STRESS for **TWO, FOUR, SIX, EIGHT** or **TEN days** at four Brine Levels.
CLIPPING WEIGHT after thirty weeks recovery.
Control=0 mS/cm E.C.=Electrical Conductivity
(EXPT. 5, GREEN SEGMENTS, NET CULTURE)

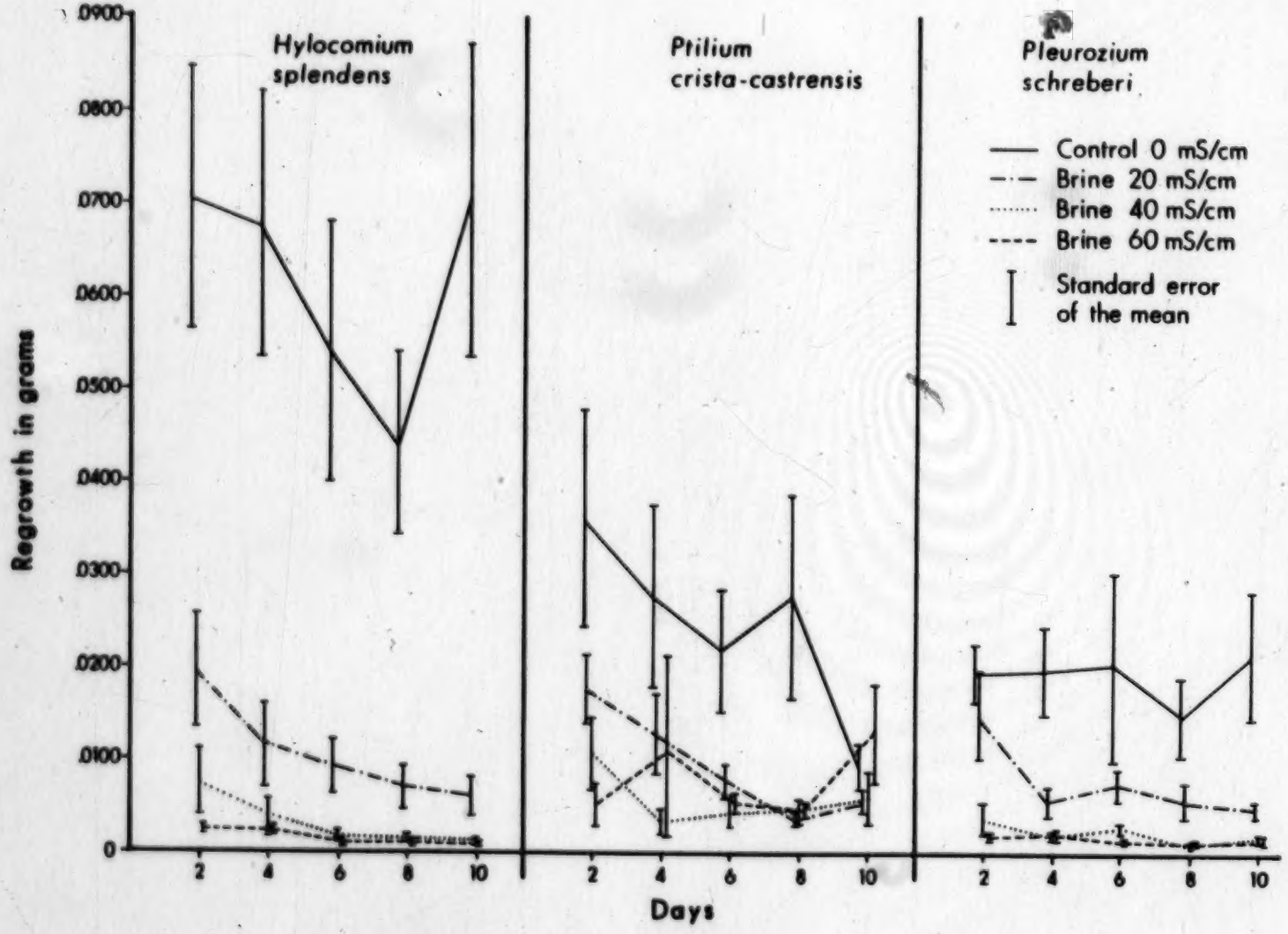

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Control 0 mS/cm
Brine 20 mS/cm
Brine 40 mS/cm
Brine 60 mS/cm
Standard error of the mean
Regrowth in grams
0900
0800
0700
0600
0500
0400
0300
0200
0100
0
2
4
6
8
10
Days

FIGURE 14: **REGROWTH vs BRINE LEVELS** of three feather mosses after BRINE STRESS at five time exposures with BRINE LEVELS of 0, 20, 40, or 60 mS/cm.
CLIPPING WEIGHT after thirty weeks recovery
Control=0 mS/cm E.C.=Electrical Conductivity
(EXPT. 5, GREEN SEGMENTS, NET CULTURE)

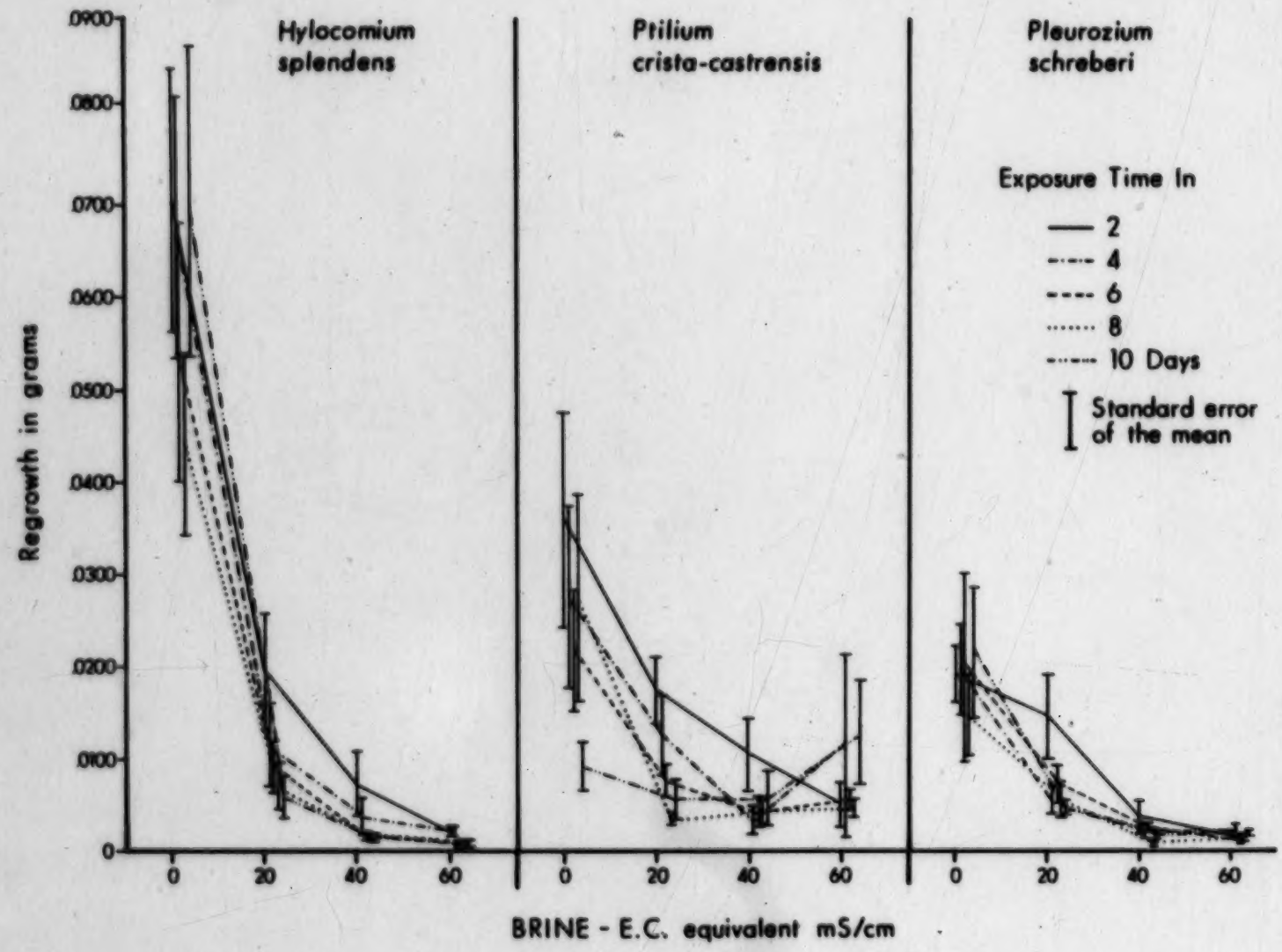

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Regrowth in grams
0900
0800
0700
0600
0500
0400
0300
0200
0100
0
0
20
40
60
Exposure Time In
2
4
6
8
10 Days
Standard error of the mean
BRINE - E.C. equivalent mS/cm

**TABLE 17: Analysis of variance, CLIPPING WEIGHT
Regrowth of three feather mosses after Brine at five Time Exposures and four Levels. Thirty week recovery.**

EXPT. 5, Green Segments, Net Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.00339	0.00068	3.58	**
Species (Spp.)	2	0.00814	0.00407	21.42	**
Brine Levels	3	0.06020	0.02007	105.63	**
Time	4	0.00252	0.00063	3.32	**
Spp. X Level	6	0.02464	0.00411	21.63	**
Spp. X Time	8	0.00084	0.00011	0.58	NS
Level X Time	12	0.00155	0.00013	0.68	NS
Spp. X Level X Time	24	0.00350	0.00015	0.78	NS
Error	295	0.05567	0.00019	1.00	
Total	359	0.16046			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

4.5 Ca/K POST FLUSH TREATMENTS-EXPERIMENT 6

4.5.1 DOES CALCIUM OR POTASSIUM AID MOSS RECOVERY?

The fourth objective was to determine if calcium or potassium or a combination of the two would aid moss recovery. Bates and Brown (1974) and Bates (1976) stated calcium was the most effective single cation to alleviate loss of intracellular potassium from mosses exposed to an artificial seawater. Calcium and potassium together were more effective than calcium alone in reducing intracellular potassium loss. In their experiments, the two elements were added to the seawater before the plants were exposed to the solution.

In Experiment 6, calcium and potassium sulfate solutions were applied after the mosses were subjected to brine of 30 mS/cm for seventy two hours. Seven levels of calcium (0 to 300 mg/L) and three levels of potassium (0 to 100 mg/L) were applied for seventy two hours of Post Flush Treatments (Figure 15, Table 18).

Differences in the variables 'Replicates' and 'Species' were highly significant ($P \leq 0.01$)(Table 18). There were no significant differences between 'Calcium' and 'Potassium' or any of the interactions. Under the given conditions of the experiment, calcium or potassium did not aid moss recovery. This does not mean that calcium or potassium are not beneficial in a spill situation. All treatments, in Experiment 6, were in solution form with no growing medium

e.g. soil, sand, peat, etc. In the field this is not the case and both plants and soil must be considered (Singh 1983). Edwards and Blauel (1975), Innes and Webster (1980), and Webster *et al.* (1983) all emphasized the importance of calcium in spill situations to prevent soil dispersion.

Net loss of intracellular potassium in *Grimmia pulvinata*, an inland moss, was almost complete within the first hour of exposure to seawater. Net uptake of sodium, in this species, occurred in the first twelve hours (Bates and Brown 1974; Bates 1976) Possibly calcium and potassium, applied after seventy two hours of brine exposure, in my Experiment 6, had little or no effect on moss recovery because the damage had already taken place.

FIGURE 15: **REGROWTH** of three feather mosses **vs Calcium levels** in Ca/K POST FLUSH TREATMENTS for three days, applied after 30 mS/cm brine stress for three days. **(Seven Calcium/Three Potassium levels)**
CLIPPING WEIGHT after thirty weeks recovery.
Control=0 mg/L calcium with 0 mg/L potassium
(EXPT. 6, GREEN SEGMENTS, NET CULTURE)

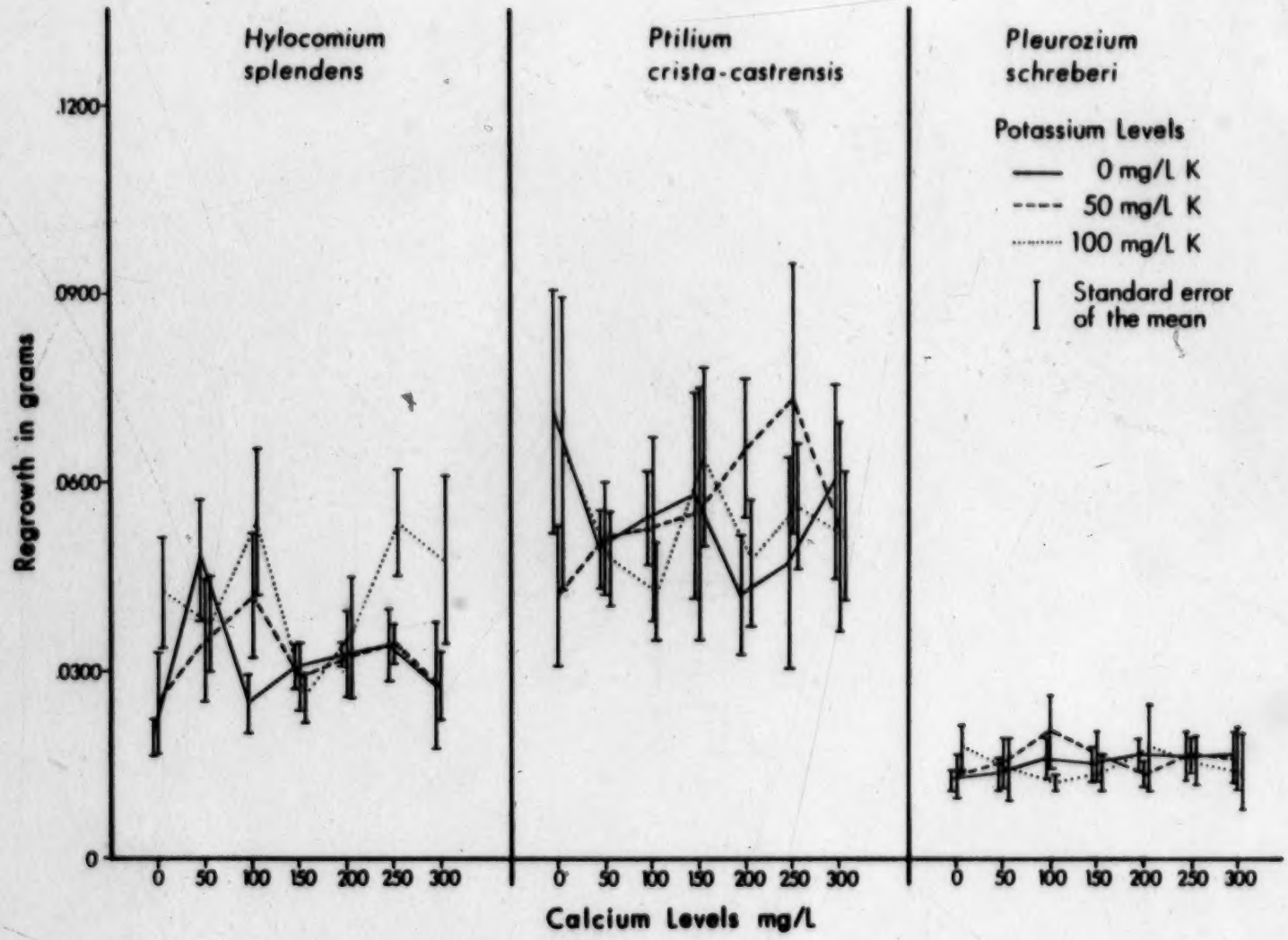

Hylocomium splendens
Ptilium crista-castrensis
Pleurozium schreberi
Potassium Levels
0 mg/L K
50 mg/L K
100 mg/L K
Standard error of the mean
Regrowth in grams
.1200
0900
0600
0300
0
0 50 100 150 200 250 300
Calcium Levels mg/L

TABLE 18: Analysis of variance, CLIPPING WEIGHT
Regrowth of three feather mosses after Ca/K Post Flush Treatments (seven Calcium and three Potassium levels) for three days, applied after 30 mS/cm brine for three days. Twenty five week recovery.

EXPT. 6, Green Segments, Net Culture

VARIABLE	DF	SS	MS	F	
Replicates	5	0.05195	0.01039	25.34	**
Species (Spp.)	2	0.09753	0.04877	118.95	**
Calcium (Ca)	6	0.00085	0.00014	0.34	NS
Potassium (K)	2	0.00074	0.00037	0.90	NS
Spp. X Calcium	12	0.00432	0.00036	0.88	NS
Spp. X Potassium	4	0.00261	0.00065	1.59	NS
Calcium X Potassium	12	0.00365	0.00030	0.73	NS
Spp. X Ca X K	24	0.00929	0.00039	0.95	NS
Error	310	0.12758	0.00041		
Total	377	0.29852			

NS=Not Significant
*=Significant (P≤0.05) **=Highly Significant (P≤0.01)

4.5.2 IS THE MOST SALT TOLERANT FEATHER MOSS *Ptilium*?

Since there were no significant differences in calcium and potassium treatments in Experiment 6 (Table 18), it can be considered a large experiment (126 replicates/species) comparing the regrowth of feather mosses after brine treatment at 30 mS/cm for seventy hours. Differences among the three species were highly significant ($P \leq 0.01$). *Ptilium* samples produced the greatest amount of regrowth of the three feather mosses (Figure 15), and its mean for the whole experiment was 0.0549 grams, compared to 0.0352 for *Hylocomium* and 0.0155 grams for *Pleurozium*. In Experiment 2 (Figure 8) there was no significant difference (standard error overlapped) between *Ptilium* and *Pleurozium* at 30 mS/cm brine. Values for all three feather mosses in Experiment 3 (Figure 12) at 30 mS/cm have overlapping standard errors but in Experiment 4 (Figure 10) there was a large difference between the response of *Ptilium* and those of *Hylocomium* and *Pleurozium*. *Ptilium* had the lowest amount of regrowth, a completely opposite result to that of Experiment 6 (Figure 15). *Ptilium* had the greatest amount of regrowth at 40 and 60 mS/cm in Experiment 5 (Figure 14). Variability of response at 30 mS/cm in Figure 14 was similar to that in Figure 15, in that *Ptilium* showed the most variability, followed by *Hylocomium* and *Pleurozium* with the least. No post flush treatments were applied in Experiment 5 (Figure 14).

Unfortunately when Experiment 6 (Figure 15) is considered a large brine experiment one important thing is absent, a distilled water (0 mS/cm) control. In the planning stages of this experiment such a control was considered but I could not determine how to randomize or analyze such a control within the factorial design of post flush treatments, without increasing treatments to an unmanageable number. Since the amount of moss available was limited, the idea was dropped. Although calcium or potassium sulfate did not aid moss recovery, under the given conditions, there was a suspicion that the two salts may have had an influence in some other way. This arose because of the response of the three mosses, *Ptilium* in particular. Three potential influences were considered: the effect of calcium, pH, and relative humidity created by the calcium and potassium sulfate solutions used in the treatments.

Crum and Anderson (1981) stated *Hylocomium* was indifferent to pH. Watson (1981) described *Pleurozium* as "strongly calcifuge, an 'indicator' of acid soil conditions." Crum (1976) defined calcifuge as "a plant not occupying calcareous habitats." Steere 1979, stated *Ptilium* occurs on steep, moist slopes of calcareous soil. If the presence of calcium or the high pH had a detrimental effect on *Pleurozium* or a favorable effect on *Ptilium* some variation from the control (0 mg/L Ca and K) to the highest concentrations of the two elements should have occurred. However *Pleurozium* showed the least variation of the three

mosses in Experiment 6 (Figure 15). There was no significant change from 0 to 300 mg/L calcium. In the case of *Ptilium*, although standard errors overlapped for 0 and 300 mg/L calcium, the means for the control were higher than those for the largest amount of calcium. If *Ptilium* was favored by the presence of calcium, one would expect the reverse to occur. That is, the highest amount of regrowth in 300 mg/L calcium and the lowest in the control. Therefore it appeared calcium did not favor *Ptilium* growth over that of *Pleurozium*. It was concluded calcium or pH were not influencing factors.

Pleurozium showed the best response to gypsum post flush treatments in my preliminary studies (Ross Daley 1980). O'Toole and Synnott (1971) found a small quantity of *Pohlia nutans*, generally considered to be calcifuge, on plots to which lime ($CaCO_3$) had been applied.

Researchers have found that drought tolerant moss species can be damaged by high humidities (Hosokawa and Kubota 1957; Proctor 1982). "*Rhacomitrium langinosum* was extremely resistant to desiccation, recovering apparently normally after 239 days' desiccation at 32% relative humidity." However this species and *Tortula ruraliformis* were most quickly damaged at the highest humidity (76%) (Dilks and Proctor 1974). Several moss species, listed by Lee and Stewart (1971), were affected by the "deleterious effects of prolonged storage in a saturated atmosphere." Prolonged storage at high humidity is not, recommended for

any moss species, especially desiccation-resistant species, as this may cause some ion leakage (Brown and Buck 1978).

Ptilium is dominant in wetter habitats than *Hylocomium* (Achuff 1974; Achuff and La Roi 1977; Lee and La Roi 1979). *Ptilium* showed the least amount of discoloration of the wet storage samples. Whether *Hylocomium* or *Pleurozium* were damaged by high relative humidities in Experiment 6 (Figure 15) was questionable. The proportion of regrowth by each species in Experiment 6 was similar to that of 20 mS/cm for four days or 40 mS/cm for two days in Experiment 5 (Figure 13). There were no post flush treatments in Experiment 5. High relative humidities in Experiment 3 (Figure 12) may have been responsible for the different response pattern of *Pleurozium* to that of *Hylocomium* and *Ptilium*. The influence of environmental factors, such as relative humidity or time of year, on moss response remains for future investigation.

In Experiment 6, *Ptilium* regrowth was greater than *Pleurozium* regrowth in all 126 replicates, no standard errors overlapped. Under the given experimental conditions, *Ptilium* appeared to be the most salt tolerant species.

4.5.3 SIGNIFICANT DIFFERENCES IN REPLICATES

The differences among the six replicates were highly significant in Experiment 6 (Table 18) as well as Experiments 1 and 5 (Tables 8 and 17). Replicates were significantly different in Experiment 3 (Table 12) Clipping Weight and Experiment 4 (Table 13) but there was no

significant difference in replicates in Experiment 2 (Table 10). There were two possible reasons for the differences in replicates. One was a bench effect in the greenhouse where environmental factors such as light, water or drainage (vials were used in Experiments 1 and 3) varied from one position to the next on the bench. An attempt was made to reduce this effect by moving the large trays to a new position on the bench every week or two but no rigid plan of rotation was followed. The second possible reason was the number of replicates was sufficiently high to detect small differences in regrowth of mosses among replications (Pinchbeck 1984, personal communication). The first reason, a bench effect, was the more likely cause of the differences among replicates.

5. SUMMARY AND CONCLUSIONS

In the six greenhouse experiments, *Hylocomium splendens*, *Ptilium crista-castrensis* and *Pleurozium schreberi* (Plates 1 to 4) were used to study the salt shock tolerance of feather mosses. All treatments were in solution form with three sources of moss samples, Swan Hills 1980, Swan Hills 1981, and Villeneuve 1981. The equipment was designed and, in some cases, manufactured by the researcher. A summary of the experiments appears at the end of Methods and Materials. An outline of procedures is given in Table 3.

Methods of culture and measurement were tested in the first four experiments. In terms of reclamation, regrowth biomass was considered the most important parameter to measure. Biomass was converted to percentages for comparisons of the three moss species on a relative basis. Net Culture (Plate 6) using green segments of moss and the Clipping Weight (Plate 5) method of weighing regrowth were preferred for these particular types of experiments.

Moss response to the various treatments was measured by regrowth biomass (Figures 4 to 15). Analyses of Variance for all six experiments, correlation of the weighing methods in Experiments 1 and 3, and percent regrowth for Experiments 2 and 4 are presented in Tables 7 to 18.

5.1 HIGHLIGHTS OF INDIVIDUAL EXPERIMENTS

The regrowth of the three species was monitored, from four to twenty five weeks at three week intervals, in Experiment 1 Weight vs Time. Some basic information on the response of feather mosses under 'ideal conditions' was the result. *Hylocomium* produced the most biomass of the three species and *Ptilium* the least. On a relative basis, the response of *Hylocomium* and *Pleurozium* was similar. Regrowth in *Hylocomium* occurred as a new increment or segment.

Experiments 2 to 4 (Brine/Sorbitol), compared the osmotic plus specific ion effects of brine to the osmotic effect of sorbitol. Five levels of brine or sorbitol (0 to 40 mS/cm equivalent) were applied for seventy two hours. Plants were then flushed with distilled water and allowed to recover. Methods of culture and type of moss sample varied in the three experiments. It was concluded the brine was more harmful to mosses than sorbitol. Mosses were more drought tolerant (osmotic effect) than salt tolerant (osmotic plus specific ion effects). *Hylocomium* was the most salt sensitive species and *Ptilium* the least drought tolerant species. Differences among the three species and between the two stresses (Brine and Sorbitol) were significant ($P \leq 0.05$) in two of the three experiments. Differences in the levels of stress were highly significant ($P \leq 0.01$) in all three experiments.

In Experiment 5 Brine-Exposure Time vs Level, plants were subjected to four levels of brine (0 to 60 mS/cm) and

five different contact times (2 to 10 days) to compare Regrowth vs Exposure Time vs Brine Level in a factorial design. The electrical conductivity (concentration) of brine was found to be more damaging to the moss than length of exposure to brine, as the variable 'Species X Level' interaction was highly significant ($P \leq 0.01$). In contrast, the 'Species X Exposure Time' interaction was not significant. Changes in regrowth biomass were minimal after six days of exposure time. Moss regrowth decreased sharply between 20 and 40 mS/cm of brine, which agreed with the results of the Brine/Sorbitol series. In most of the Brine/Sorbitol experiments (Experiments 2 to 4) regrowth in brine decreased between 30 and 40 mS/cm.

Twenty one treatments of calcium or potassium sulfate were tested in Experiment 6, Ca/K Post Flush Treatments, to determine if these two salts would aid moss recovery. In Experiment 6, mosses received a salt shock of 30 mS/cm brine for the standard seventy two hours. Plants were then flushed with distilled water, a standard treatment used throughout the six experiments. Seven levels of calcium (0 to 300 mg/L) and three levels of potassium (0 to 100 mg/L) were then applied for seventy two hours. Differences among species were highly significant ($P \leq 0.01$) in Experiment 6. *Ptilium* gave the highest amount of regrowth followed by *Hylocomium* and *Pleurozium*. Under the given conditions, there were no significant differences in the calcium and potassium treatments. They did not aid moss recovery significantly in

this experiment. This does not imply that calcium or potassium are not beneficial under field conditions. All experiments, including Experiment 6, used treatments in solution form therefore no growing medium e.g. soil, sand, peat, etc. was considered. Both plants and soil must be considered (Singh 1983). The benefits of gypsum applied to salt spill sites are reported by Clark and Thimm (1975); Edwards and Blauel (1975); Webster (1975); Innes and Webster (1980a b) and Webster *et al.* (1983).

5.2 FEATHER MOSS RESPONSE PATTERNS

5.2.1 REGROWTH

Hylocomium produced the greatest and *Ptilium* the least amount of regrowth of the three feather mosses when brine or sorbitol was absent. The amount of *Hylocomium* regrowth was three or four times more than that of *Ptilium* or *Pleurozium*, which compared favorably with weight ratios of orginial moss segments. Regrowth in *Hylocomium* occurred as a new segment or increment. This agreed with Busby's (1976) comment that cyclic patterns in this species were caused by abrupt changes of environmental conditions rather than changes of season.

In *Ptilium* and *Pleurozium*, new growth appeared mainly as an extension of the apical tip, with a few lateral shoots, when the original moss material was in a vertical position (Bag Culture). When the original moss material was

horizontally positioned (Net Culture) regrowth of the three species occurred at 90°. Plant regrowth in Net Culture was similar to that found in the field whereas regrowth segments were somewhat elongated in Bag Culture. Shoots growing through the bag and sticking to the bag resulted in loss of biomass in the Clipping Weight of *Ptilium* and to a lesser extent *Pleurozium*. Bag Culture was unsatisfactory for growing *Ptilium*. In contrast *Hylocomium* was well suited to Bag Culture if the 'starching effect', of concentrated solutions such as brine or sorbitol, was avoided.

Moss samples from Swan Hills 1980 (Figures 8 and 9) showed a reduced vitality compared to samples from Villeneuve 1981 (Figures 4 and 10). Brown segments of moss showed a similar reponse pattern to that of green segments of moss.

5.2.2 TOLERANCE

Ptilium appeared to be the most salt tolerant species, especially at higher concentrations of brine. I say this with some caution because relative humidity or time of year may have influenced moss response. *Ptilium* gave the greatest amount of regrowth in Experiment 5 where 60 mS/cm was the highest brine level and Experiment 6 where all plants were stressed for seventy two hours at 30 mS/cm.

Hylocomium was the least salt tolerant species. This agreed with results of a field study by Hettinger (1980). Large decreases in regrowth occurred in brine treatments of

0 to 10 mS/cm (Figure 10). This pattern was repeated in a later experiment (Figures 13 and 14) where *Hylocomium* regrowth decreased greatly from 0 to 20 mS/cm. Differences in *Hylocomium* regrowth between these levels takes on added significance if one considers that regrowth of this species was three to four times the amount of *Ptilium* or *Pleurozium* in Experiment 1 (Figure 4).

In Experiment 3 however, there was no significant difference between *Hylocomium* regrowth at 0 and 10 mS/cm in the brine treatments. Experimental procedure was probably responsible for the atypical response of all three feather mosses (Figure 12) in this experiment. In comparison with moss response in similar experiments (Tables 14 to 16), effects of sorbitol and brine on *Hylocomium* and *Ptilium* were reduced in Experiment 3. The effect of sorbitol on *Pleurozium* was increased in Experiment 3. Unlike the other brine/sorbitol experiments, bags and moss were dried and held at 80% relative humidity to obtain a 'before' recovery weight in Experiment 3.

One could speculate that high relative humidity influenced the moss response in this experiment. Proctor (1982) stated "a moist break of twenty four hours allowed complete recovery of surviving cells from the effects of a preceding dry period in *Hylocomium splendens*." Another possibility was that *Hylocomium* and *Ptilium* were more tolerant than *Pleurozium* to salt stress followed by drought stress. *Pleurozium* showed its variable character with

different response patterns from experiment to experiment, but generally response was between that of *Hylocomium* and *Ptilium*. However, regrowth of *Pleurozium* was signifcantly greater than that of *Ptilium* at 20 mS/cm brine (Figure 8) and 0 to 30 mS/cm brine (Figure 10). *Pleurozium* gave the least amount of regrowth of the three species in Experiment 6, where the brine concentration was 30 mS/cm.

Ptilium was the least drought tolerant moss (Figures 8 and 10), which agreed with the findings of Achuff (1974), and Achuff and La Roi (1977). They found the habitat of *Ptilium* was the wettest of the three feather mosses. *Ptilium* discolored the least under 'wet' storage. Drought tolerant species of moss are damaged by high relative humidities (Hosokawa and Kubota 1957; Lee and Stewart 1971; Dilks and Proctor 1974; Proctor 1982). Taking this into consideration, *Hylocomium* may be the most drought tolerant feather moss because it discolored more quickly than the other two species under conditions of high humidity. Sorbitol treatments (Figure 10) did not have a marked effect on *Hylocomium*. Also regrowth of *Hylocomium* did not decrease, compared to *Ptilium* and *Pleurozium*, at twenty two weeks recovery (Figure 4) when increased temperatures and probable decreased humidities occurred. Lee and La Roi (1979) found that *Hylocomium* had a wider tolerance of moisture regimes, both xeric and hydric, than *Ptilium* and *Pleurozium*. La Roi (1984, personal communication) commented that *Hylocomium* was found in areas above the timberline whereas *Ptilium* and

Pleurozium were absent.

5.3 FUTURE STUDIES

The environmental impact of various chemical products used in the recovery of petroleum is an area for future research. Past attempts at burying chemcial wastes by the 'out of sight, out of mind' approach have had a bad habit of 'resurfacing' years or decades later. An expanded investigation of phytotoxic effects of brine is suggested since the potential for future salt spills is great. Increased volumes of water, aging of water handling systems and using old equipment which was not designed to handle the present technological procedures increase the potential for brine spills (Haynes *et al.* 1978; Skinner 1981).

Why calcium was ineffective in aiding moss recovery in this series of experiments could be investigated further with varied times and methods of application. An experiment to determine the effectiveness of calcium to aid moss recovery in the field would also be desireable.

Moss physiology and reclamation of forested areas with native species are relatively new areas of interest that require future study. Do environmental factors such as humidity or time of year affect moss response patterns to salt stress or post flush treatments? The increase in *Ptilium* regrowth at 60 mS/cm (Figure 14) was rather intriguing. A similar increase in regrowth of moss occurred in my preliminary experiments, therefore it may warrant

further investigation. A public media source presented a hypothesis that high saline solutions increased regeneration of cell tissues, however I did not investigate the possibility further. A laboratory method to compare salt tolerance of bryophytes to that of vascular plants would be helpful but because of the varying physiology of the two, may not be possible.

The brine spill reclamation plots (Innes and Webster 1980a; Webster *et al.* 1983), that began my interest in moss/salt relations, could be used for a study of plant succession after a salt spill. The history is well documented and the two sites represent two different forest communities.

There was a certain link between *Ptilium* and colonization by invaders. The fine, closely spaced leaves of *Ptilium* (Plates 1 and 3) provided a moist, flat surface for moss spores. Most of the colonizing species were so called 'weedy species' (Vitt 1982, personal communication). *Pohlia nutans* and *Aulacomnium palustre* were the two main species of invaders. In terms of field application, colonization by moss spores and regeneration of moss fragments may be the most significant idea that arose from the unwelcomed arrival of invaders in these experiments. A brine spill site could be 'seeded' with pulverized moss material by adding it to the last water applied in the flushing operations. This idea has yet to be tested in the field, however Bayfield (1976) compared such a technique on several different greenhouse

media. Mixtures of species fragments were more successful than those of one or a pair of species.

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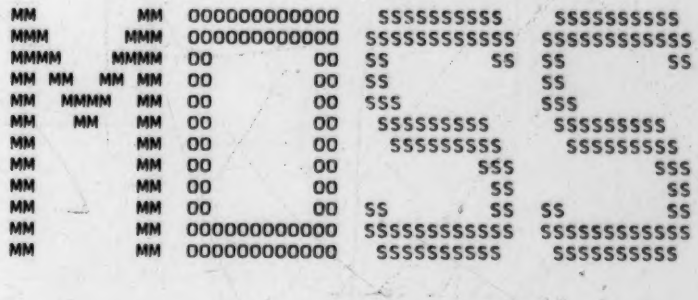
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